DVS Sciences

CyTOF® 2 Mass Cytometer

User Manual

CyTOF2 Mass Cytometer User Manual

This is a Class A device and is for use in commercial, industrial or business environments.

Warning: This is a Class A product. In a domestic environment this product may cause radio interference, in which case the user may be required to take adequate measures.

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Preface

This manual provides:

- An overview of the CyTOF®2 instrument and technology.
- Instructions for calibration, operation, data acquisition and maintenance.
- Troubleshooting recommendations.
- Safety recommendations for operation of the instrument

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Chapter 1
Introduction to CyTOF® 2 and Mass Cytometry

The CyTOF® 2 mass cytometer analyzes individual cells labeled with stable heavy metal isotopes using state of the art Time-of-Flight Inductively Coupled Plasma mass spectrometry (TOF ICP-MS) technology (Figure 1.1). With over 120 detection channels, the CyTOF® 2 has the exquisite ability to simultaneously resolve multiple elemental probes per cell at high acquisition rates without the need for compensation, thereby maximizing the per-cell information obtained from a single sample. These attributes provide researchers with an unparalleled ability to generate high resolution phenotypic and functional profiles of cells from normal and diseased states.

Figure 1.1 The CyTOF® 2 Mass Cytometer.

Principles of Mass Cytometry

Mass cytometry employs elemental tags that have higher molecular weights than those elements that are naturally abundant in biological systems. The CyTOF® 2 is specifically designed to measure these high mass elemental tags on a per-cell basis.

Cells stained with metal conjugated probes in a single cell suspension are introduced into the CyTOF® 2. The cells undergo a multi-step process within the instrument, resulting in generation of a file that records the identity and amount of each probe on each cell (Figure 1.2).

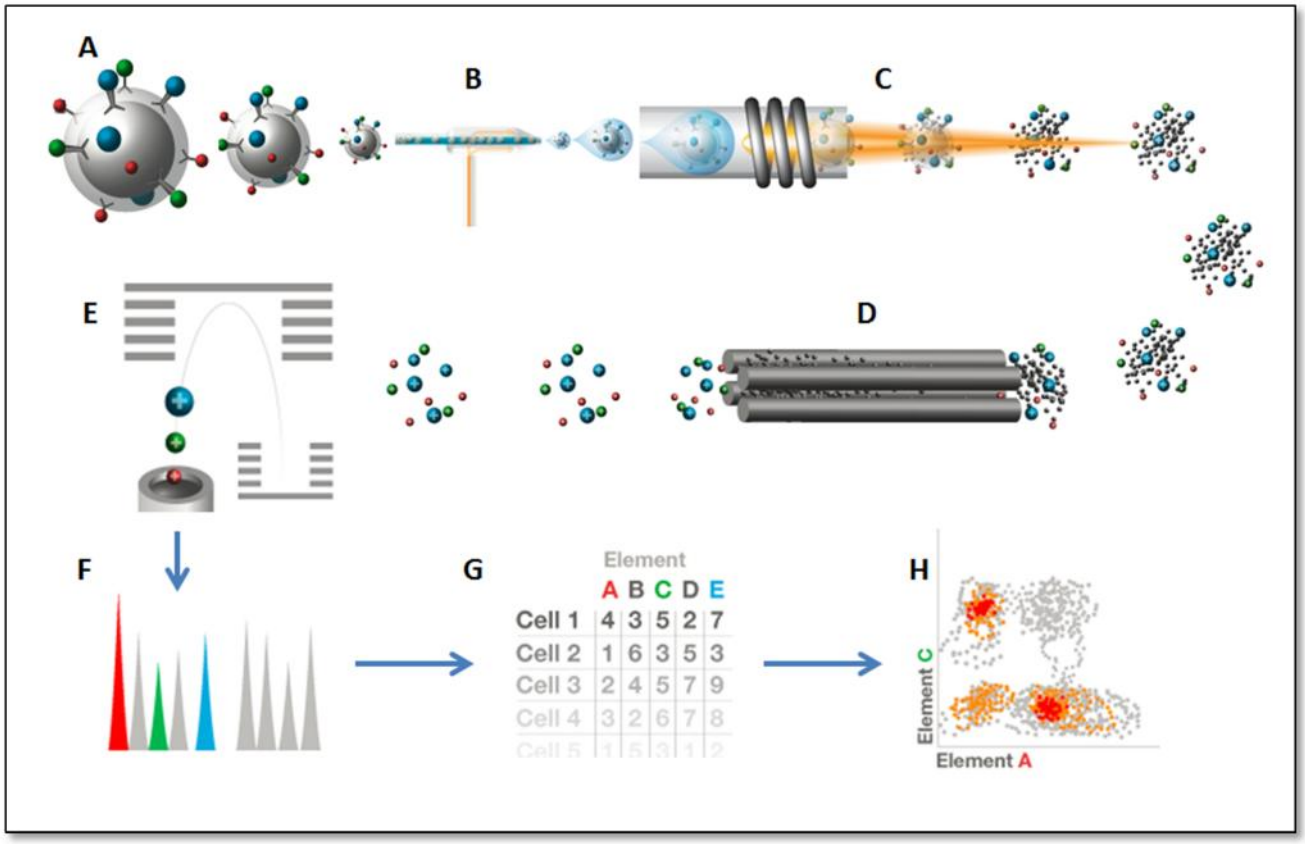


Figure 1.2 Mass Cytometry Workflow.

A liquid sample containing cells labeled with heavy metal isotope conjugated probes (A) is introduced into the nebulizer (B) where it is aerosolized. The aerosol droplets are directed into the ICP torch (C) where the cells are vaporized, atomized and ionized. Low mass ions are removed in the RF Quadrupole Ion Guide (D), resulting in a cloud of ions enriched for the probe isotopes. The ion cloud then enters the Time-of-Flight (TOF) chamber (E) where the probes are separated on the basis of their mass to charge ratio as they accelerate towards the detector. The time-resolved detector thus measures a mass spectrum (F) that represents the identity and quantity of each isotopic probe on a per-cell basis. Data is generated in .fcs format (G) and analyzed in third-party software programs (H).

A schematic of the instrument is shown in Fig 1.3, divided by color to indicate the major steps of mass cytometry workflow. Each of these steps is described in detail in the following section.

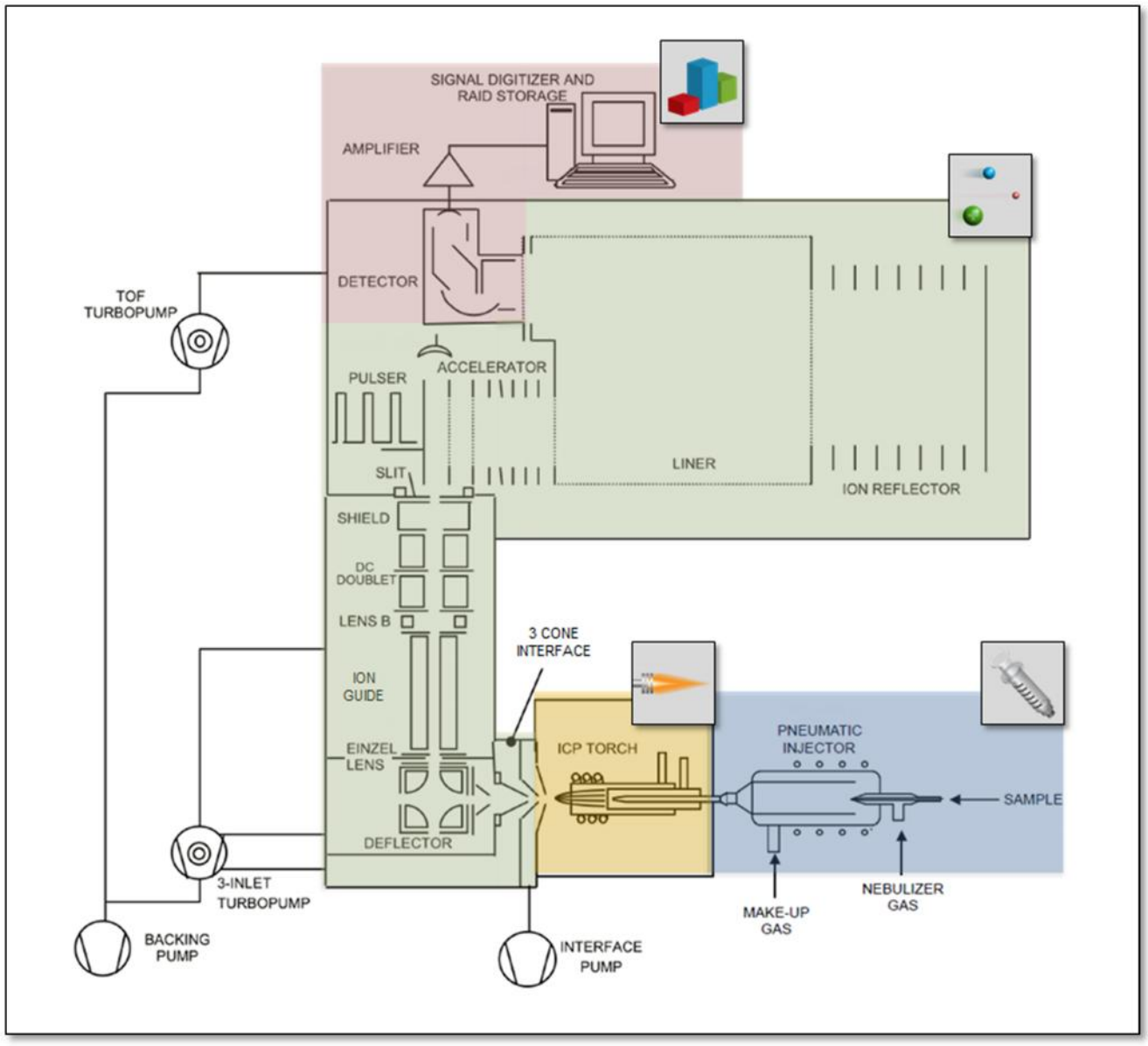


Figure 1.3 CyTOF2 schematic. Mass Cytometry workflow is divided into sample introduction (blue), ionization (yellow), mass analysis (green), and data acquisition (red).

Sample Introduction

The sample introduction system de-solvates the liquid sample suspension and introduces cells one at a time into the ICP source for ionization (Fig 1.4). The liquid sample is introduced (manually via syringe or automatically via Autosampler) into a nebulizer where it is aerosolized into a heated spray chamber. Within the spray chamber, the high temperature partially vaporizes the aerosol, and gas flows direct the aerosolized cells to the ICP source. These steps are described in detail below.

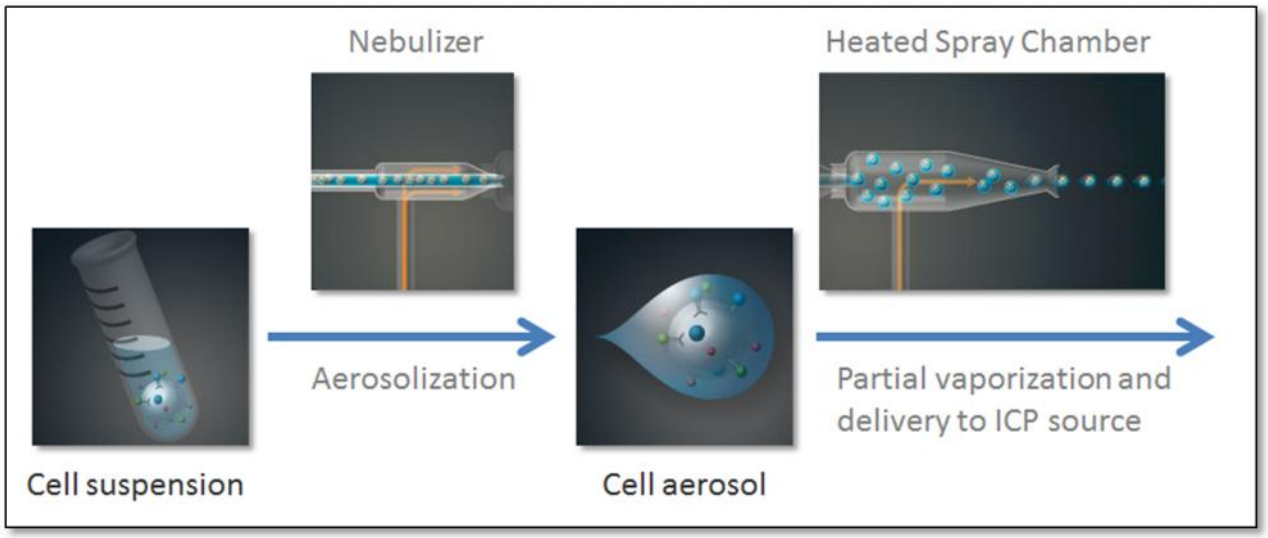


Figure 1.4 Sample Introduction. The liquid sample suspension is syringe-injected, then aerosolized by the nebulizer into the spray chamber, which partially vaporizes the aerosol and delivers it to the plasma.

Delivery of sample to the nebulizer

Liquid cell suspensions are introduced into the instrument manually using a syringe or automatically using an Autosampler.

Manual Introduction

The manual sample introduction system upstream of the nebulizer is composed of the sample syringe, syringe drive, flow injection valve, dual sample loop system, waste vessel, and carrier fluid vessel (Figure 1.5). First, the initial sample is loaded into a 1 mL syringe and injected through the sample loading port into one 500 µL loop of tubing of the dual sample loop system. During this step, the flow injection valve is rotated to open a fluidic pathway from the sample syringe through the sample loop and out to the waste vessel. Thus, any sample in excess of 500

μL is lost to the waste vessel circuit. Once the sample is loaded into the loop, the flow injection valve rotates, opening a fluidic pathway from the syringe drive to the sample loop to the nebulizer. Then the syringe drive pushes carrier fluid through the fluidic circuit, delivering the sample to the nebulizer. The syringe drive controls the volumetric flow rate, and is typically operated at 45 μL/min.

A couple of special features of the system optimize sample throughput by minimizing time between samples. First, the syringe drive automatically recharges with carrier fluid when it is low by drawing from the carrier fluid vessel, thereby eliminating the need to manually recharge the pump. Secondly, the dual sample loop system allows washing of the alternate sample loop during data acquisition from sample in the first loop.

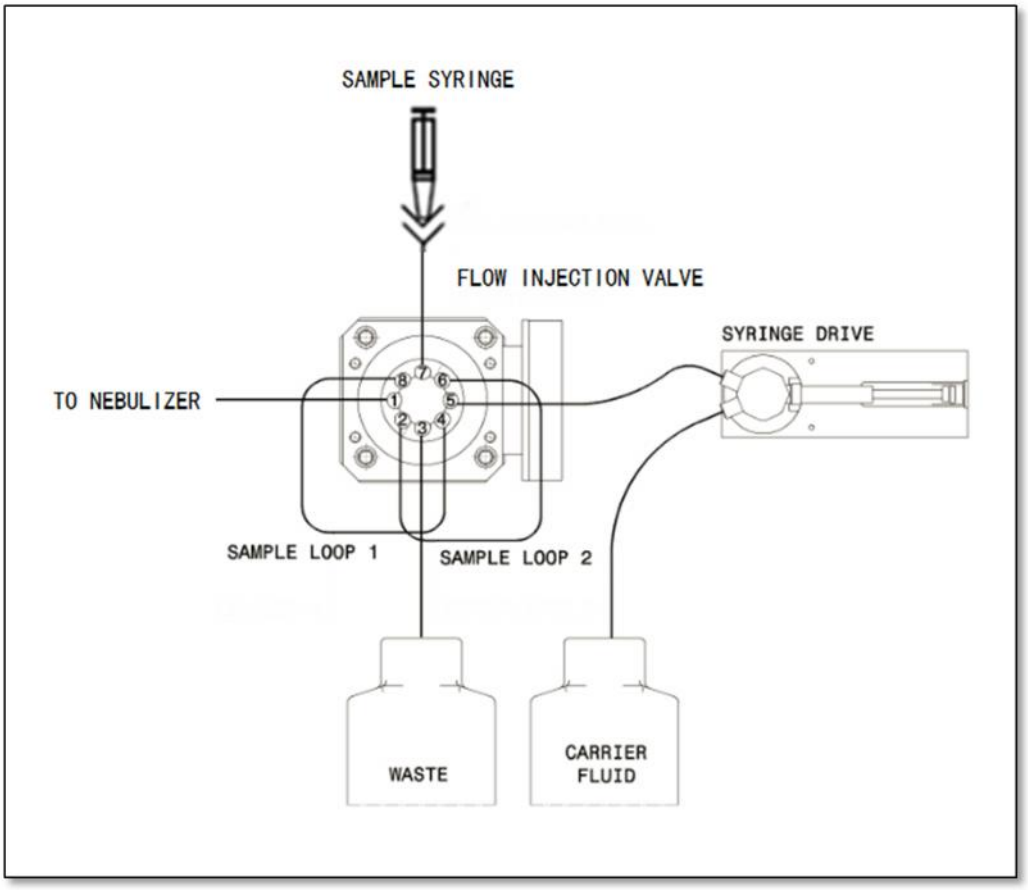


Figure 1.5 Schematic of Sample Introduction System upstream of the nebulizer.

Autosampler

If the CyTOF2 is connected to the Autosampler (Fig. 1.6), samples loaded into 96-well plates are automatically introduced into the system, allowing unattended instrument operation and sample data acquisition. The autosampler contains a separate dedicated liquid sampling automation system that is described in detail in the CyTOF Autosampler Manual.

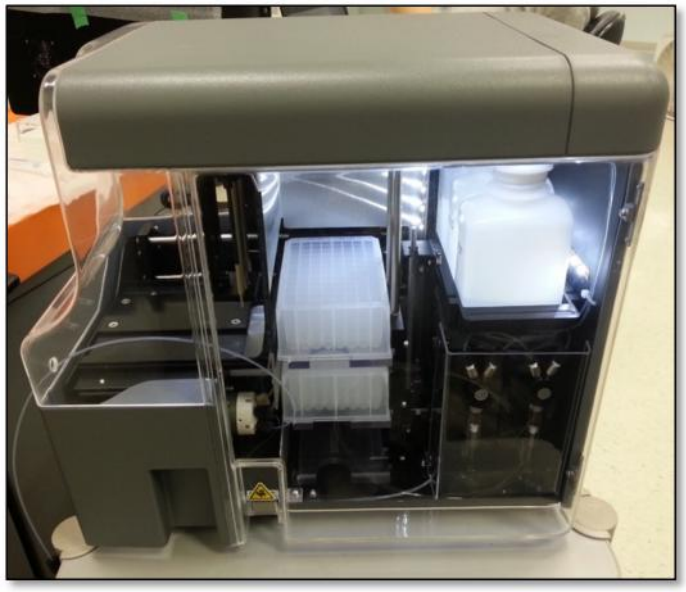


Figure 1.6 Image of the AS-5 autosampler.

Delivery of de-solvated sample aerosol to the ICP source

For liquid sample analysis, it is critical to remove as much water as possible from the sample so that it can be efficiently ionized in the plasma. This is achieved first by aerosolizing the sample in the nebulizer followed by delivery of heated aerosol to the plasma by the spray chamber (Fig 1.7)

Nebulizer

The CyTOF® 2 employs a glass concentric nebulizer consisting of an inner capillary that carries the liquid sample and an outer chamber that carries argon gas flow (called nebulizer gas). Both liquid (at 45 uL/min) and gas (at 0.15-0.35 L/min) flows are directed towards the spray chamber through a tapered end (Fig. 1.8). Because the liquid chamber has a small inner diameter, the sample velocity is high and pressure is low within the nebulizer, and as the sample exits the tip, concentric pressure exerted by the exiting nebulizer gas breaks it up into a fine-droplet aerosol.

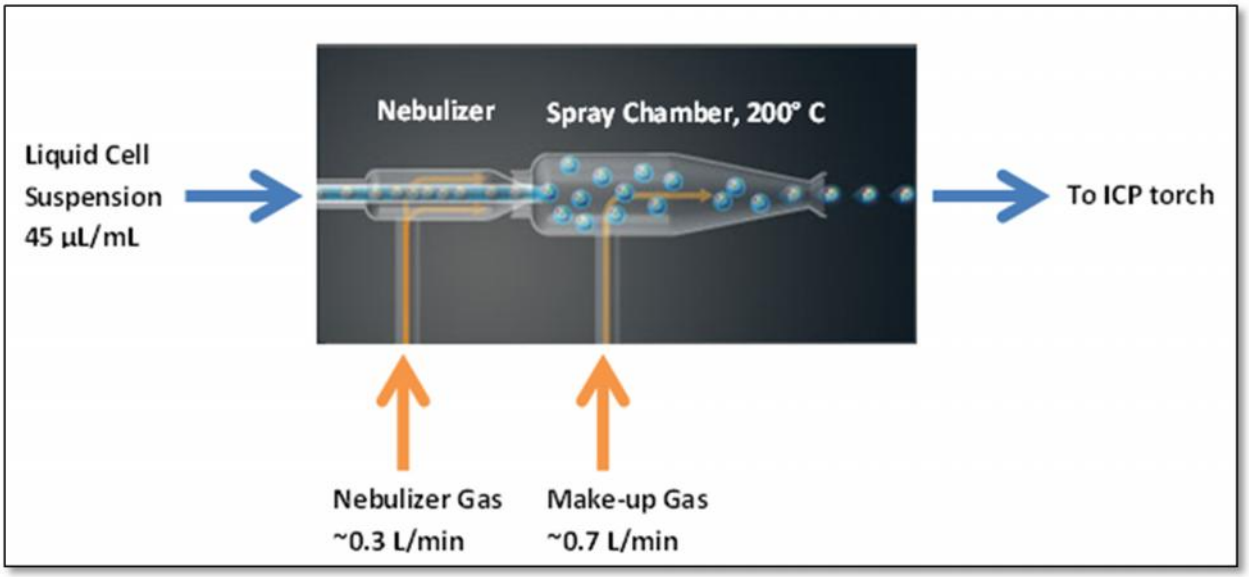


Figure 1.7 Sample aerosolization and delivery to the ICP torch. Liquid cell suspension is aerosolized by nebulizer gas as it exits the nebulizer. Make-up gas carries the aerosol through the heated spray chamber where it is partially de-solvated and delivered to the ICP torch.

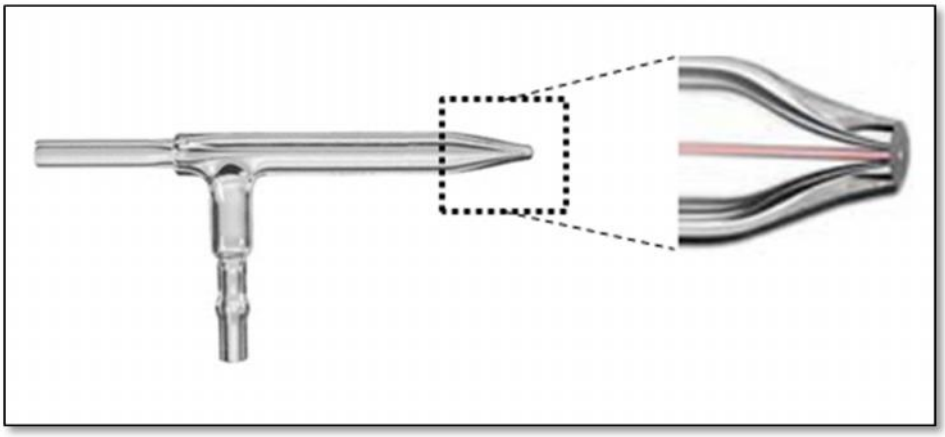


Figure 1.8 Nebulizer. Liquid sample enters from the left and argon Nebulizer gas from the bottom. Sample chamber narrows into a capillary, pulling liquid rapidly to the tip (enlarged, at right, with liquid sample indicated in red) where shear forces exerted by accelerated nebulizer gas break the liquid into aerosol droplets.

Spray Chamber

The aerosolized sample exits the nebulizer directly into the spray chamber, which is housed within a 200˚C heating block. Argon gas (called 'make-up' gas) is pumped into the spray chamber (~0.7 L/min), and this high flow of heated gas partially vaporizes the sample to minimize condensation for optimal ionization as it directs the aerosol to the ICP source.

Ionization

The mixture of single cell aerosol droplets and argon that exits the spray chamber is transmitted to the ICP source where it is successively vaporized, atomized and ionized in the plasma for subsequent mass analysis (Fig 1.9). The formation and characteristics of the plasma responsible for the ionization process are described below.

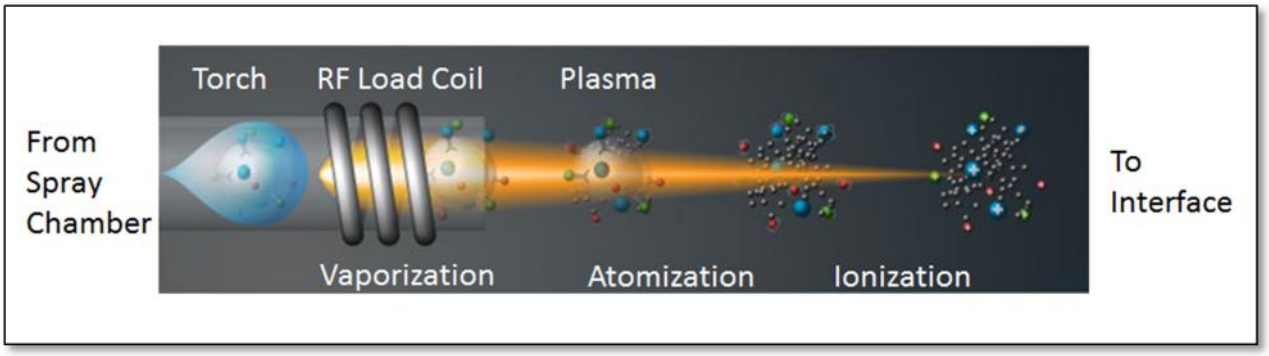


Figure 1.9 Electromagnetic energy generated by the RF load coil surrounding the quartz torch sustains argon plasma (orange) that vaporizes, atomizes, and ionizes individual cell aerosols from the spray chamber. The positive ion component of the cell-derived plasma cloud enters the ion optics and mass analyzer chambers of the CyTOF2 through the interface.

Plasma Torch

The plasma is created within the plasma torch by induction using a radio-frequency-generated electromagnetic field. The torch consists of the torch body – a fused assembly of two concentric quartz tubes – and a quartz sample injector tube that is inserted inside the torch body. When assembled, the torch consists of three concentric chambers. The outermost chamber (between the torch body tubes) contains argon 'plasma' gas flowing at 17 L/min that is ignited to form the plasma. The central chamber (between the inner torch body tube and the sample injector) contains argon 'auxiliary' gas flowing at ~1 L/min that is used to change the position of the base of the plasma relative to the sample injector. The innermost chamber inside the sample injector transmits the argon stream and sample aerosol from the spray chamber directly into the center of the plasma. The torch assembly is mounted inside an induction load coil that is supplied with radio-frequency generated current that creates an electromagnetic field.

Formation of the ICP discharge and ionization of the sample

Plasma, the fourth state of matter consisting of charged particles, is formed by collision-induced ionization of argon gas within an intense electromagnetic field. First, argon plasma gas flows tangentially from the outer chamber of the torch body. RF power supplied to the load coil produces an oscillating current (40 MHz), creating a strong electromagnetic field precisely at the point the plasma gas exits the outer chamber. A high voltage spark strips away free electrons from the exiting argon atoms. These free electrons accelerate dramatically in the electromagnetic field and collide with sufficient energy to ionize the argon gas into plasma. Temperatures within the plasma typically range from 5,000 to 10,000K. When the aerosolized sample is introduced through the injector into the base of the plasma, the water droplets are rapidly vaporized. The de-solvated individual cells are then broken down into a cloud of ground-state atoms. Subsequent electron collisions result in ionization of the cell. Thus, the argon ionic beam that exits the plasma contains bursts of ionic clouds corresponding to individual cells that were introduced into the torch in aerosol form (Fig. 1.10).

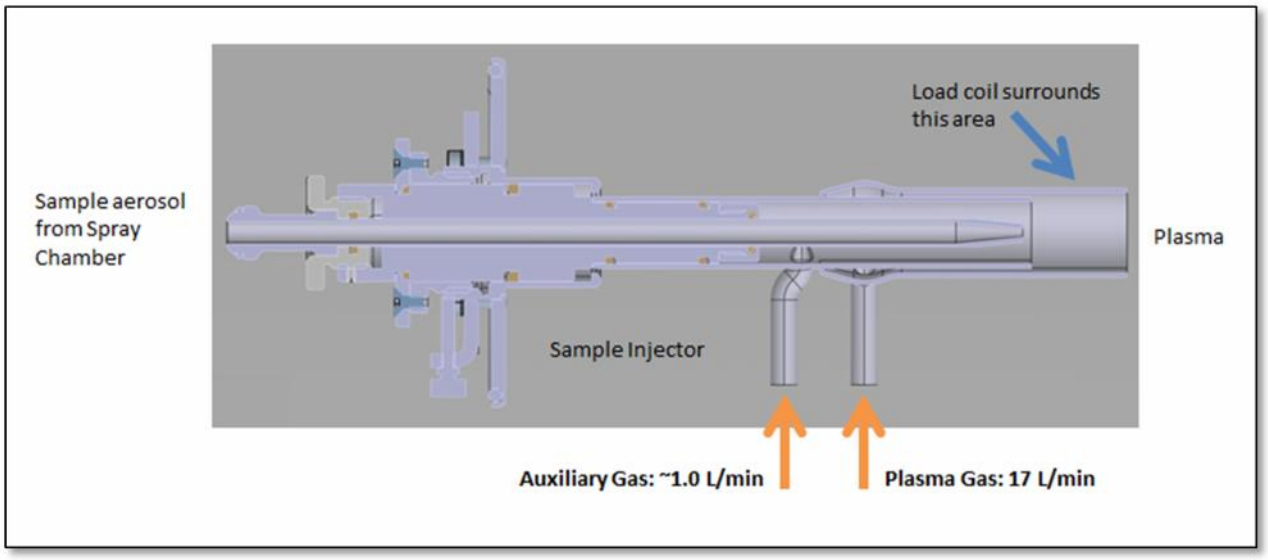
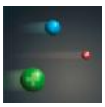


Figure 1.10 Cross-section of the CyTOF®2 plasma torch.



Mass Analysis

The ion beam exiting the plasma contains a heterogeneous mixture of argon ions, endogenous cellular ions, isotopic probe ions, neutral particle, and photons. The beam travels through the interface region into a series of low vacuum chambers that contain ion optics to eliminate unwanted materials and the time-of-flight mass analyzer to separate the isotopes of interest for downstream quantification and data analysis (Fig 1.11).

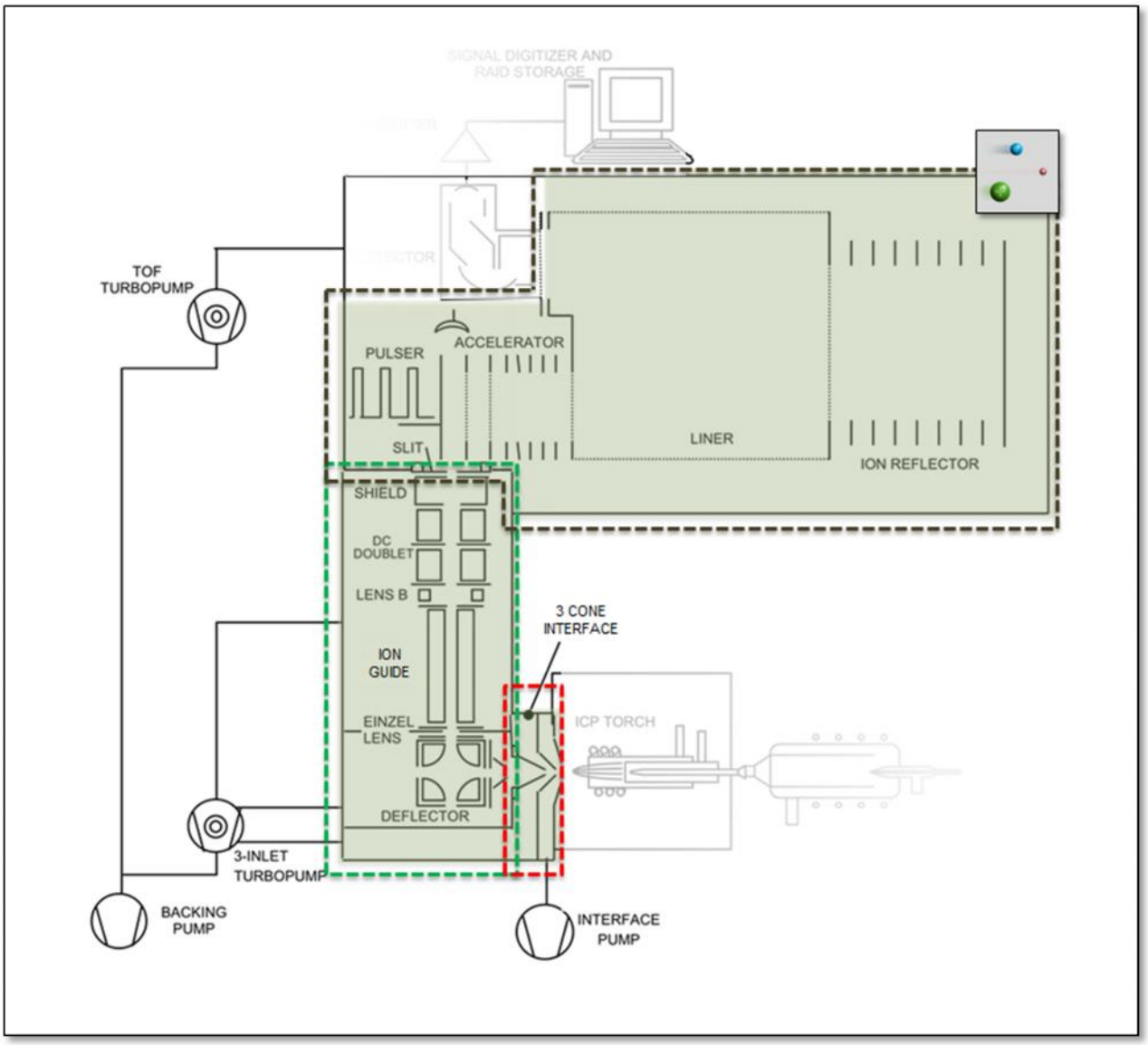


Figure 1.11 CyTOF2 ion optics. The ion beam leaving the torch enters the low pressure ion optical chamber (green) through the 3-cone interface (red). High mass ions leaving the Quadropole Ion Guide are directed to the Time-of-Flight chamber (black) where they are separated on the basis of mass to charge ratio and directed to the detector.

Interface Region

In order to analyze the identity and amount of isotopic probes in each cell derived cloud, the high temperature ion beam exiting the plasma at atmospheric pressure (760 Torr) passes through a series of ion focusing and separating chambers. These require low vacuum to eliminate any collisions with gas molecules on the pathway to the detector. To achieve this, the plasma is sampled through an interface region that dramatically reduces the temperature and pressure of the incoming ionic clouds (Fig. 1.12).

The purpose of the interface region is to efficiently transport ions from the high temperature plasma at atmospheric pressure to the room temperature chambers that house the ion optics at less than 10^{-3} Torr. The CyTOF2 uses a three-cone interface to transport the ionic beam into a low pressure vacuum: sampler (1.1 mm diameter orifice), skimmer (1 mm) and reducer (1.2 mm). All three cones are made of nickel, and the interface housing is water-cooled to dissipate the significant heat generated by the plasma. The rapidly expanding ionic clouds exiting the plasma enter the sampler cone orifice into the sampler-skimmer region, which is pumped by a 40 m^3/h rotary pump to 2.3-2.5 Torr. The ions then pass through the skimmer cone to the skimmer-reducer region, which is pumped by the 25 L/s stage of the 3-stage turbo-molecular pump to 2-4x10^{-2} Torr. Finally, the ions pass through the reducer cone which serves not only to reduce the pressure (300 L/s stage of the 3-stage pump de-pressurizes the chamber to 3-5x10^{-4} Torr). The ions that emerge from the reducer cone are accelerated and focused by an electrostatic field defined by the potentials of the reducer and a downstream conical lens, and the subsequent highly focused beam is propagated to the ion optics and mass analyzer.

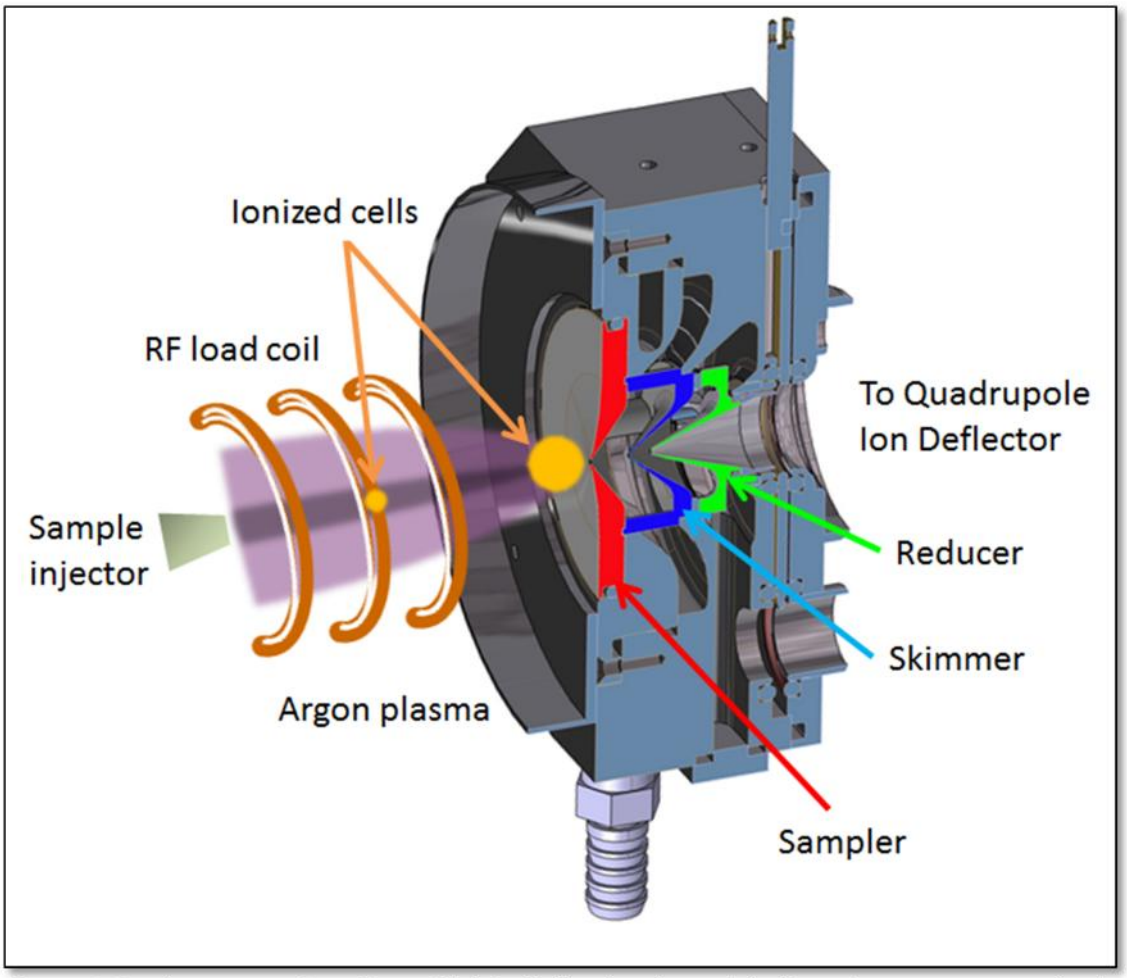


Figure 1.12 The vacuum interface which includes the three nickel interface cones: sampler (red), skimmer (blue) and reducer (green).

Quadrupole Ion Deflector

The beam propagating through the reducer contains some non-ionized material and photons in addition to ions. If not filtered, neutrals can attach to instrument components resulting in signal drift, and photons that reach the detector are registered erroneously as ions. To eliminate these problems, the beam passes perpendicularly through an electrostatic quadrupole ion deflector, which turns positively charged ions towards the downstream ion optics, while neutrals and photons follow an undisturbed pathway into the turbo molecular pump.

RF Quadrupole Ion Guide

The pure ionic beam leaving the quadrupole ion deflector is dominated by low mass ions that are not of analytical interest (H+, C+, O+, N+, OH+, CO+, O2+, Ar+, ArH+, ArO+) and that are of such high abundance that they would quickly damage the detector. To remove these ions, the beam is focused via an Einzel lens and directed into the RF-only Quadrupole Ion Guide (Fig.

1.13). The four rods of the quadrupole are supplied with alternating current (AC), with opposing pairs of rods always having the same AC charge that alternates based on the radio frequency setting. Low mass ions (m/z<80) gyrate dramatically and are ejected from the central path of the quadropole, while high mass ions are focused (ie guided) through this pathway. For optimal mass filtration performance, the Ion Guide chamber is pumped by the 400 L/s stage of the 3-stage turbo-molecular pump to 2-5 x 10^{-6} Torr. As a result, a stream of burst events (corresponding to individual cells) that contain only the high molecular weight isotopic probes exits the RF Quadrupole Ion Guide.

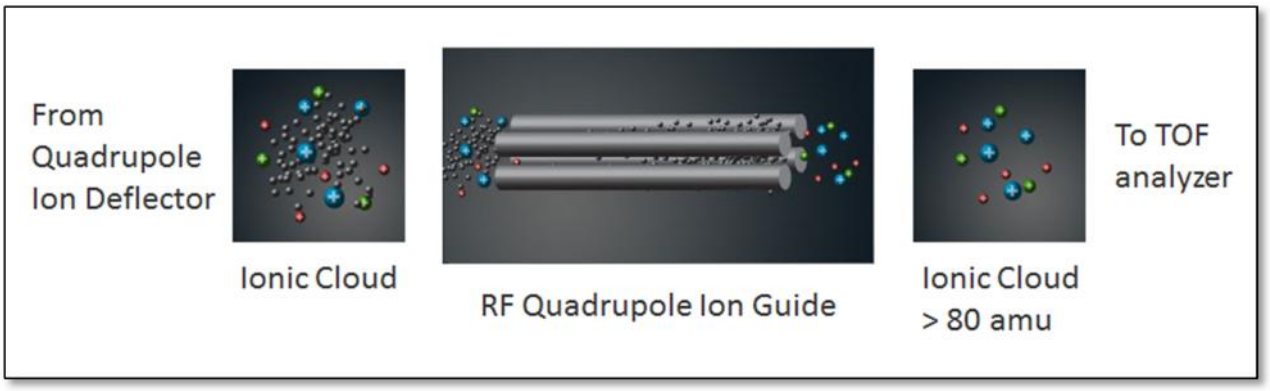


Figure 1.13 The Quadrupole Ion Guide removes unwanted low molecular weight argon and endogenous cellular ions from the beam that emerges from the quadrupole Ion Deflector, transmitting clouds that contain isotopic probe ions (>80 amu) to the TOF analyzer.

Time-of-Flight Mass Analyzer

The burst event ion clouds that exit the Ion Guide consist of a mixture of high molecular weight probes in a randomly distributed array. These ions are then sent to the orthogonal-acceleration reflectron Time-of-Flight (TOF) mass analyzer, which separates the probe ions on the basis of the mass to charge ratio (Fig. 1.14).

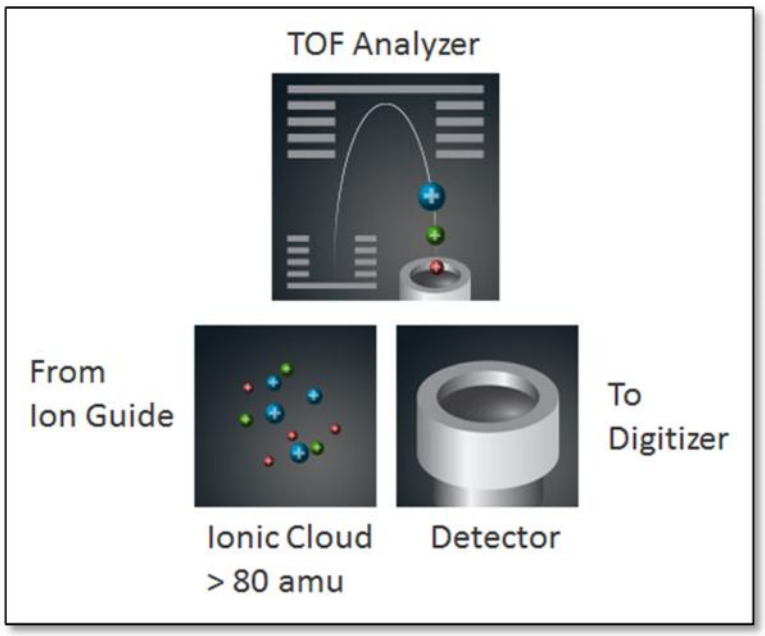


Figure 1.14 Separation of ions in the TOF chamber. Ionic clouds are subjected to an electrostatic force that orthogonally accelerates the incoming ions toward the detector. As a result, the ions separate based on their mass/charge ratio, with lighter elements reaching the detector first.

The cylindrical beam exiting the Ion Guide first passes through the DC Quadrupole Doublet, which flattens the beam so that it can enter through the rectangular entrance slit into the accelerator chamber of the TOF analyzer (maintained at 10^{-6} Torr by the TOF turbo-molecular pump). At 13 μs intervals (frequency of 76.8 kHz), a pulse of several hundred volts is applied to the push out plate, accelerating the accumulated packet of ions orthogonally toward the reflector, which redirects the ions toward the detector. The electric fields in the accelerator and reflector are configured to focus ions of into tight time-resolved bands regardless of initial position or energy. The relationship between time of ion flight to the detector and their m/z is:

$$t = t_0 + A\sqrt{\frac{m}{z}},$$

in which t_0 and A are derived from the mass calibration procedure. Because the isotopes used for probes in mass cytometry have the same charge, each packet of ions resolves into a series of bands, with the lightest probes reaching the detector first and each successively heavier mass reaching the detector at a later time interval. Each time resolved band of ions of mass M is separated from its M+/- 1 neighbor by 20-25 ns.

After the first packet of ions is pushed out and detected, a second pulse pushes out the next packet of ions for detection and the cycle repeats until data acquisition is complete.

Vacuum system

The mass analysis system requires high vacuum to prevent random collisions of ions with gas molecules as they travel to the detector. As described in the various sections above, the CyTOF2 employs a 5-stage differential pumping system to sequentially drop the pressure from 760 Torr outside the interface to 10^{-6} Torr in the TOF chamber (Table 1.1). The system includes the interface pump for the Sampler-Skimmer chambers; a three-stage turbo-molecular pump for the Skimmer-Reducer chamber (stage 1), the Deflector chamber (stage 2) and the Ion Guide chamber (stage 3); and the TOF turbo-molecular pump for the TOF chamber.

Under standard conditions the 5-stage vacuum system of the CyTOF® 2 instrument operates at the five pressure ranges detailed in the table below.

Table 1.1 CyTOF2 vacuum system

Vacuum Pump		Chamber	Pressure (Torr)
Interface		Sampler-Skimmer	2.3-2.5
	Stage 1 – 25 L/s	Skimmer-Reducer	2-4 x 10^{-2}
Turbo-molecular, 3-Stage	Stage 2 – 300 L/s	Deflector	3-5 x 10^{-4}
	Stage 3 – 400 L/s	Ion Guide	2-5 x 10^{-6}
Turbo-molecular, TOF		TOF	0.3-1.5 x 10^{-6}

Data Acquisition

This section describes the process whereby the ions organized by mass in the TOF chamber are detected, converted into digital values and analyzed (Fig. 1.15).

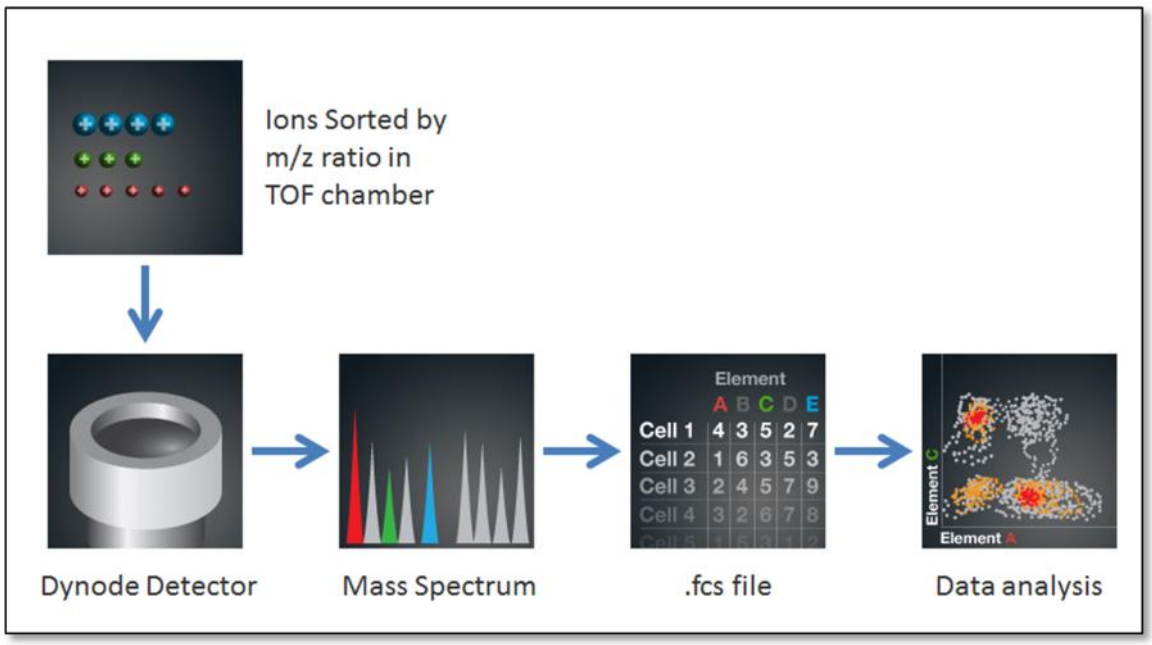


Figure 1.15 Detection of ions and data analysis workflow.

Detector

The ions separated in the TOF chamber are detected using a discrete dynode electron multiplier. When an ion strikes the first dynode of the detector, several secondary electrons are liberated. These electrons strike the next dynode where they generate more electrons. This process is repeated at each dynode, resulting in an electron pulse that is captured by the anode of the detector. The output analog signal is amplified and converted by a dual-8-bit digitizer to digital values at 1 ns sampling intervals. The digitizer trigger delay dictates the first mass channel to be recorded per push while the segment length dictates the mass range to be recorded per push. Instruments are set to collect data from at least 120 mass channels (each corresponding to 1 amu), typically starting at mass 88.

Dual Count Scale

CyTOF resolves multi-element samples using time-of-flight, with ions from each isotope arriving at the detector centered in discrete 20-25 ns time windows (within each 13µs push) depending on their mass to charge ratio. At very low particle concentrations, the probability of pulse

signal overlap is negligible, and particle count is most precisely determined by simply counting the number of pulses (i.e. Pulse Count, Fig. 1.16, left). As particle concentration increases, ion pulses begin to arrive at the detector at the same time. In this situation, pulse count underestimates the true ion count, and integrated intensity becomes a more accurate measurement (Fig 1.16, right).

The range of data that CyTOF collects requires collection of Dual Data, which means that Pulse Count and Intensity values are collected for every channel. CyTOF plots the entire data range on a single Dual Signal scale, the units of which are actual counts of particles that hit the detector. To achieve this, two things are done. First, a Dual Count Coefficient is applied which converts analog Intensity into actual counts according to the following formula:

Counts = Intensity X Dual Count Coefficient

Second, a dual switchover threshold is applied, below which Pulse Count is used and above which counts from coefficient-converted analog Intensity is used. Using the dual count scale, CyTOF2 quantifies bound particles per cell across a wide range of signal input.

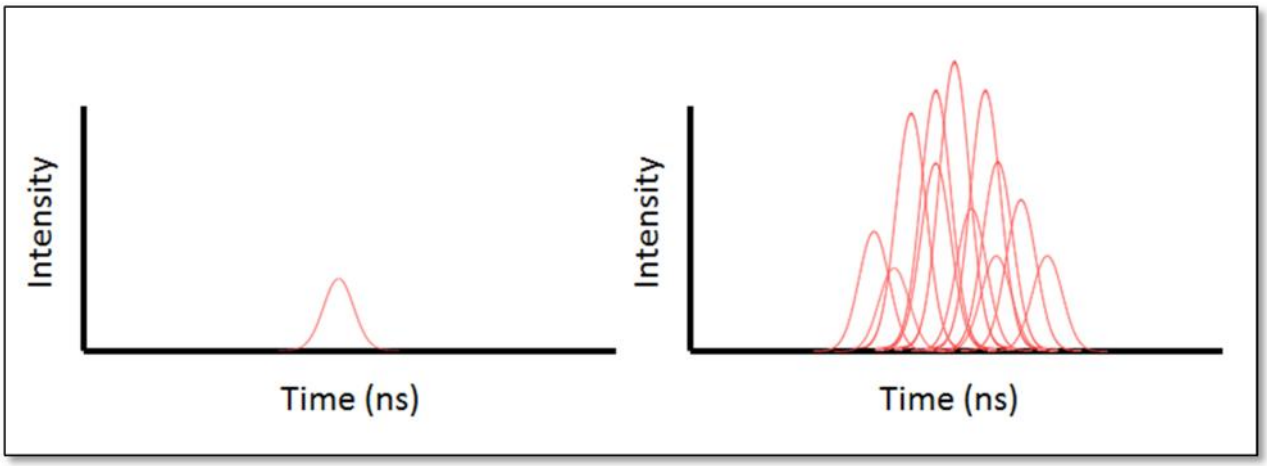


Figure 1.16 Impact of analyte concentration on signal measurement. At low analyte concentration (left), pulses do not overlap. Because each pulse delivers a different number of electrons to the anode and therefore different intensity values, it is more precise to count pulses when ion concentration is very low. Here the pulse count is 1. At higher analyte concentrations (right), pulses overlap, and counting pulses will underestimate the true number of particles that hit the detector. Here the pulse count is 8 (if we count discernible peaks) even though 16 ions hit the detector. Thus, at high analyte concentration, it is more accurate to use integrated intensity, and convert this intensity value to counts using a calibration coefficient.

Cell Detection and Acquisition Data File format

Data for each 13μs push is digitized sequentially and integrated to obtain mass peaks for the channels selected for analysis. The resulting record is processed according to cell event selection criteria set by the user. These criteria (described in detail in Chapter 6) include a

minimum signal threshold and a range for event duration consistent with single cell events. As a result, the data acquired contains the integrated number of total ion counts for each selected analyte on a per-cell basis. These data are saved as text (.txt) and flow cytometry standard (.fcs) 3.0 format for data analysis in compatible software programs.

Chapter 2
Preparing Your Laboratory for the CyTOF® 2 Mass Cytometer

This chapter is designed to help you to understand the CyTOF® 2 mass cytometer instrument and conditions required for successful installation of the instrument in your laboratory. The CyTOF® 2 mass cytometer is shipped to you as a complete system with the exception of the following items which must be obtained prior to installation: electrical power, exhaust vents, and argon gas supply with approved regulator.

When preparing the laboratory for instrument installation by a DVS Sciences Field Service Engineer, the following items must be considered:

- System layout
- Electrical requirements
- Argon gas requirements
- Exhaust ventilation
- Environmental conditions

System Layout

Figure 2.1 CyTOF® 2 mass cytometer (B) and components including the chiller (A), computer (C) and monitor (D).

The CyTOF® 2 system consists of the main instrument, a refrigerated chiller (Polysciences cat# 6105PE) and a system computer with workstation (Fig. 2.1). The dimensions of the instrument, chiller and optional autosampler are given in Table 2.1. Note that the autosampler is designed to rest on the instrument shelf and so does not occupy an additional lab space. The system computer may be placed on a bench or a separate computer table.

Table 2.1 Dimensions of CyTOF®2, Chiller, and Autosampler

Component	Width (cm/in)	Height (cm/in)	Depth (cm/in)	Weight (kg/lb)
CyTOF®2	97/38	132/52	79/31	285/628
Chiller	38/15	64/25	67/27	81/178
Autosampler *	39/16	24/10	36/14	20/44

* Autosampler is optional, and when installed, rests on the instrument shelf and therefore does not take up any additional lab space

It is recommended that the instrument be located near the required electrical and gas supplies as well as the coolant supply. The CyTOF® 2 mass cytometer is on wheels and can be moved for service and regular maintenance. It is recommended that you leave a space of at least 30 cm (12 in) behind the instrument to provide adequate clearance for the vent hoses. Also, allow space (approximately 50 cm / 20 in) on the right side of the instrument for access to circuit breakers. Access for most service procedures is through the front of the instrument. The front and rear vents of the chiller must be a minimum of 24 inches (61 cm) away from walls or vertical surfaces so air flow is not restricted.

Electrical Requirements

Power to the CyTOF®2 instrument is to be delivered from two 30 A single-phase 200-240 V AC, 50-60 Hz dedicated electrical branch circuits (Table 2.2). The electrical supply requirements and approximate power consumption of the major accessories and options are summarized in Table 2.3. If the power line is unstable, fluctuates, or is subject to surges, additional control of the incoming power may be required.

60-Hertz-Operation Connections

The instrument is shipped with two 400 cm line cord cables. The installation kit includes two NEMA L6-30 plugs (250 V, 30 A) for use with two 60 Hz single phase outlets. The instrument is wired for power at the time of installation.

50-Hertz-Operation Connections

The instrument is shipped with two 380 cm line cord cables. It is up to the service person installing the instrument to wire the cables with the appropriate plugs. The single phase connectors must be supplied by the customer.

Connections to a three-phase power

Connection to a three-phase power may be required (by local electrical code). The instrument can be connected to two phases and to the ground wire of the three phase line. The three-phase plugs must be supplied by the customer.

Table 2.2 CyTOF® 2 Instrument Power Specifications

Power Consumption	
Maximum Volt Amperes (total, both circuits)	9000 VA
Maximum Continuous Current (per circuit)	20 A
Voltage Specification	
Operating Voltage	200-240 V AC
Maximum Allowable Percent Sag	5 %
Maximum allowable Percent Swell	5 %
Phase (singe or three)	Single or between two of the three phases
Frequency Specifications	
Operating Frequency	50 or 60 Hertz
Allowable Frequency Range	+- 1Hz
Waveform Specification	
Maximum Supply Voltage Total Distortion	5 %
Maximum Supply Voltage Distortion by Single Harmonic	3 %

Table 2.3 Electrical Requirements of Accessories

Equipment	Voltage (AC)	Power
Chiller	Plugged into CyTOF	Plugged into CyTOF
Autosampler (optional)	100 -240 V	100 VA
Computer	100-240 V	1050 VA

Argon Gas Requirements

Argon is used as the ICP torch gas with the CyTOF® 2 system. The quality criteria for argon are listed below.

Purity	≥ 99.996%
Oxygen	< 5 ppm
Hydrogen	< 1 ppm
Nitrogen	< 20 ppm
Water	< 4 ppm

Argon gas at 80±1 psi (522±7 kPa) can be supplied to the CyTOF® 2 system from liquid or gas storage tanks. The choice of liquid argon or gaseous argon tanks is determined primarily by the availability of each and the usage rate.

Safe Handling of Gas Cylinders

The permanent installation of gas supplies is the responsibility of the user and should conform to local safety and building codes. The following are a list of safety precautions that should be observed when handling argon gas cylinders.

- Fasten all gas cylinders securely to an immovable bulkhead or a permanent wall.
- When gas cylinders are stored in confined areas, ventilation should be adequate to prevent dangerous accumulations. Move or store gas cylinders only in a vertical position with the valve cap in place.
- Locate gas cylinders away from heat or ignition sources, including heat lamps. Cylinders have a pressure relief device that will release the contents of the cylinder if the temperature exceeds 52 °C (125 °F).
- When storing cylinders external to a building, the cylinders should be stored so that they are protected against temperature extremes (including the direct rays of the sun) and should be stored above ground on a suitable floor.
- Gas cylinders should be clearly marked to identify the contents and status (e.g. full, empty).
- Do not attempt to refill gas cylinders.
- Use only approved regulators and hose connectors. Left-hand thread fittings are used for fuel gas tank connections whereas right-hand fittings are used for oxidant and support gas connections.
- Arrange gas hoses away from foot traffic to avoid damage.
- Perform periodic gas leak tests by applying a soap solution to all joints and seals.

Exhaust Ventilation

The CyTOF 2 instrument generates heat and argon gas during operation. These must be exhausted from the system.

Exhaust venting is important for the following four reasons:

- To protect laboratory personnel from ozone and hot argon generated in plasma.
- To minimize the effects of room drafts and laboratory atmosphere on ICP torch stability.

- To help protect the instrument from corrosive vapors that may originate from samples.
- To remove dissipated heat which is produced by the ICP torch, ICP power supply and pump motors.

Vent Positions

The CyTOF® 2 instrument has two separate vents, both of which are located at the back of the instrument (Figure 2.2).

The Torch Box Vent exhausts plasma and the vacuum pump system, and removes fumes and vapors from the torch housing. It is 9.7 cm (3.8 in) from the right side of the instrument when viewed from the rear and 110.6 cm (43 in) above the floor.

The System Vent exhausts heat from the blower that cools the roughing pumps, system power supply and ICP generator. It is 68 cm (26.8 in) from the left side of the instrument when viewed from the rear and 34.6 cm (13.6 in) above the floor

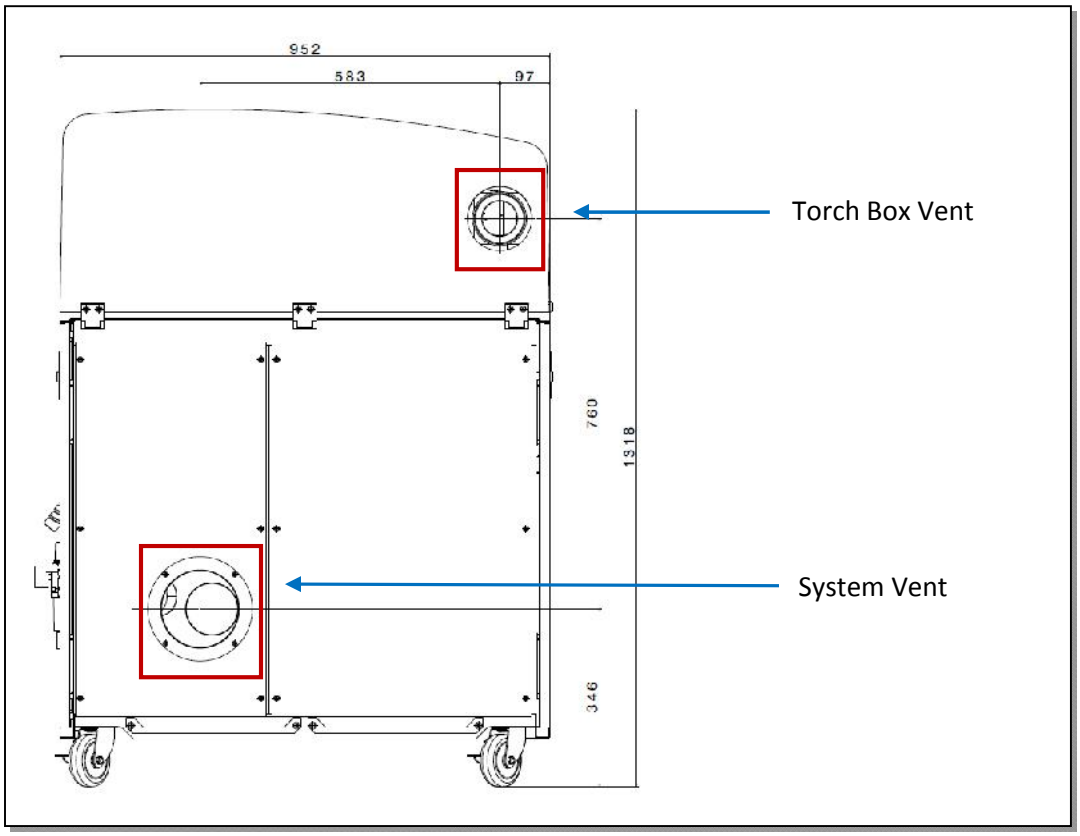


Figure 2.2 Instrument rear view schematic drawing with vent positions shown.

Flow Rate

The main 100-mm (4-in) venting system must provide a flow rate of approximately 70 L/sec ± 10% (150 ft^3/min). The second, 150-mm (6-in) venting system must provide a flow rate of approximately 210 L/sec ± 10% (450 ft^3/min). In addition to the accuracy of +- 10% requirement, the flow through the 100 mm (4 in) duct should also be stable to within +- 10 %, both in the short-term (during one 5 min experiment) and long-term (through a day). We recommend a 100 mm (4 in) ID torch box exhaust hose and a 150 mm (6 in) ID ICP Power Supply/Roughing Pump air exhaust hose. The CyTOF® 2 instrument is supplied with 3 m (10 ft) of 100-mm (4 in) and 3 m (10 ft) of 150-mm (6 in) flexible hoses (Table 2.4). A venting system that uses a single inlet duct, having a flow rate of 280 L/sec (600 ft^3/min), should be divided into the two separate 100 mm (4 in) and 150 mm (6 in) ducts equipped with individual dampers. Ensure that there is access to the dampers during installation.
The flow rates as measured with the hoses connected to the ducts will need to be verified and adjusted during installation of the instrument. The static pressure drop caused by the CyTOF® 2 system is 1.2 inches H_2O (200 pascals).

Table 2.4 Venting specifications

Vent	Hose Diam. mm (in)	Flow Rate L/s (ft^3/min)	Anemometer m/s (ft/min)	Vented Outside Lab Power W (BTU/hr)
Torch Box	100 (4)	70 (150)	9 (1695)	200 (690)
System	150 (6)	210 (450)	11.5 (2250)	2800 (9400)

Venting System Recommendations

The exhaust flow rate at the instrument (the ability to vent the system) is dependent on the blower provided by the customer, the duct length, material and the number of elbows or bends used. If an excessively long duct system or a system with many bends is used, a stronger blower may be necessary to provide sufficient exhaust volume at the instrument.

Additional recommendations on the venting system include:

- The duct casing and venting system should be made of materials suitable for temperatures as high as 70 °C (160 °F) and be installed to meet local building code requirements.
- Locate the blower as close to the discharge outlet as possible. All joints on the discharge side should be airtight.
- Equip the outlet end of the system with a backdraft damper.

- Take the necessary precautions to keep the exhaust outlet away from open windows or inlet vents and to extend it above the roof of the building for proper dispersal of the exhaust.

- Equip the exhaust end of the system with an exhaust stack to improve the overall efficiency of the system.

- For best efficiency, make sure the length of the duct that enters into the blower is a straight length at least ten times the duct diameter. An elbow entrance into the blower inlet causes a loss of efficiency.

- Provide make-up air in the same quantity as is exhausted by the system. An airtight laboratory can cause an efficiency loss in the exhaust system.

- Ensure that the system is drawing properly by placing a piece of cardboard over the mouth of the vent

Environmental Conditions

The CyTOF® 2 mass cytometer has been designed for indoor use only. The environment in which the instrument is installed should meet the following conditions:

- **Room Temperature** - The room temperature should be between 15 and 30 °C (59 and 86 °F) with a maximum rate of change of 2.8 °C (5 °F) per hour.

- **Relative Humidity** – The relative humidity should be between 20 and 80%, non-condensing.

- **Elevation** - The instrument should not be operated at an elevation greater than 2,000 m (6,500 ft) above sea level. Use of the instrument at elevations greater than 2,000 m is subject to acceptance by local inspection authorities.

The instrument should be located in an area that is:

- Free of smoke and corrosive fumes

- Not prone to excessive vibration

- Out of direct sunlight

- Away from heat radiators

WARNING: Do not use the instrument in an area where explosion hazards may exist.

Table 2.5 Instrument specifications summary

Gas	Argon (≥99.996 Purity)	345 7kPa (80± 1 psi)	20 L/min
Coolant (Filtered)	Glycerol and DIW	3.8 L/min	345 14 kPa (50± 2 psi)
Electrical Power	Maximum Voltage		9000 VA
	Operating Voltage		200-240 V AC
	Operating Frequency		60 Hz
Exhaust Vents (Open)	4” (Torch Box)	70 L/s	150 ft /min
	6” (Electronics)	210 L/s	450 ft /min

Chapter 3
Instrument Interface

This chapter contains annotated figures of the CyTOF® 2 instrument.

Figure 3.1 CyTOF® 2 Front View.

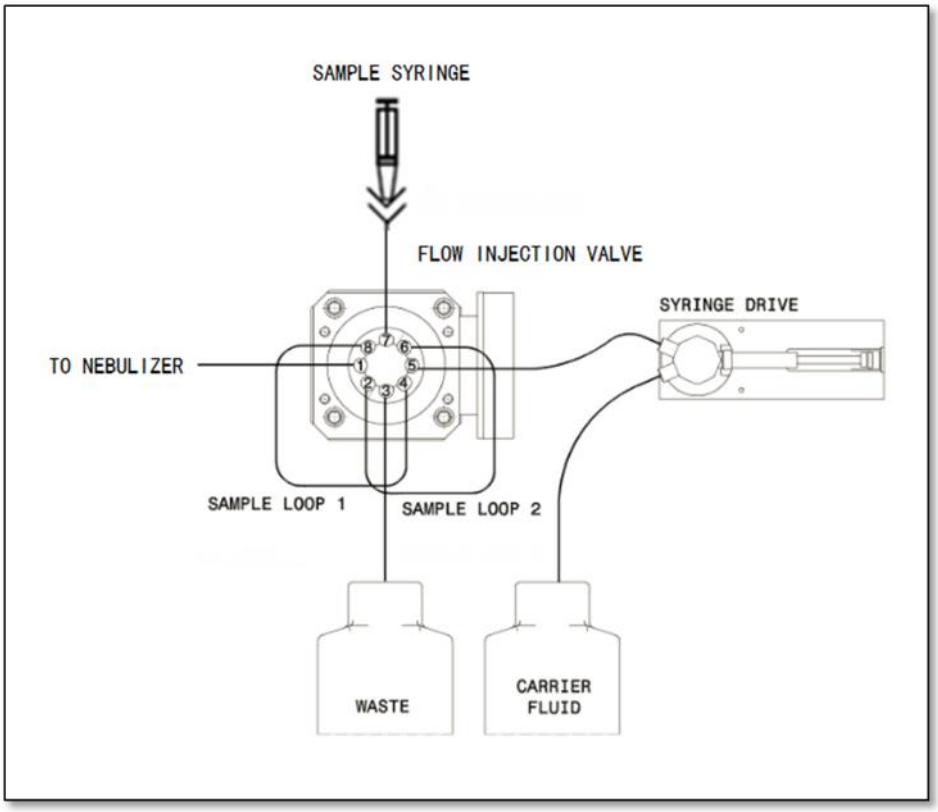


Figure 3.2 Sample Introduction System Schematic.

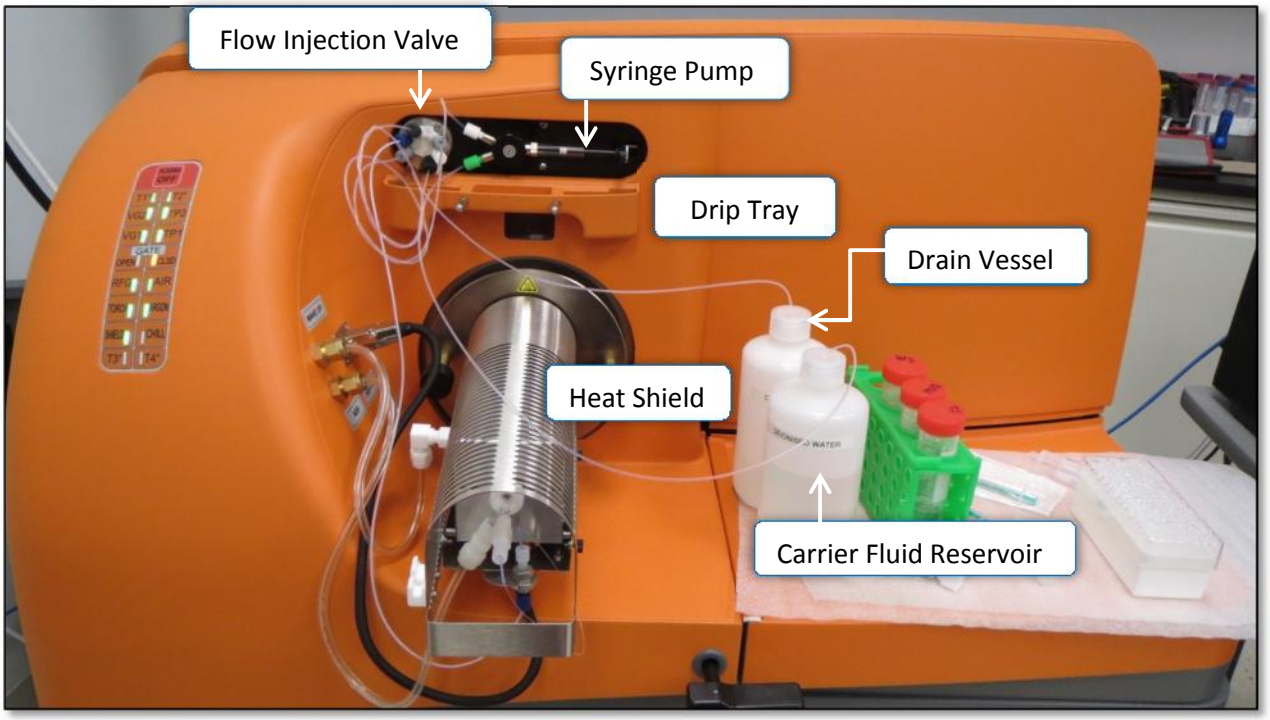


Figure 3.3 Sample Introduction System.

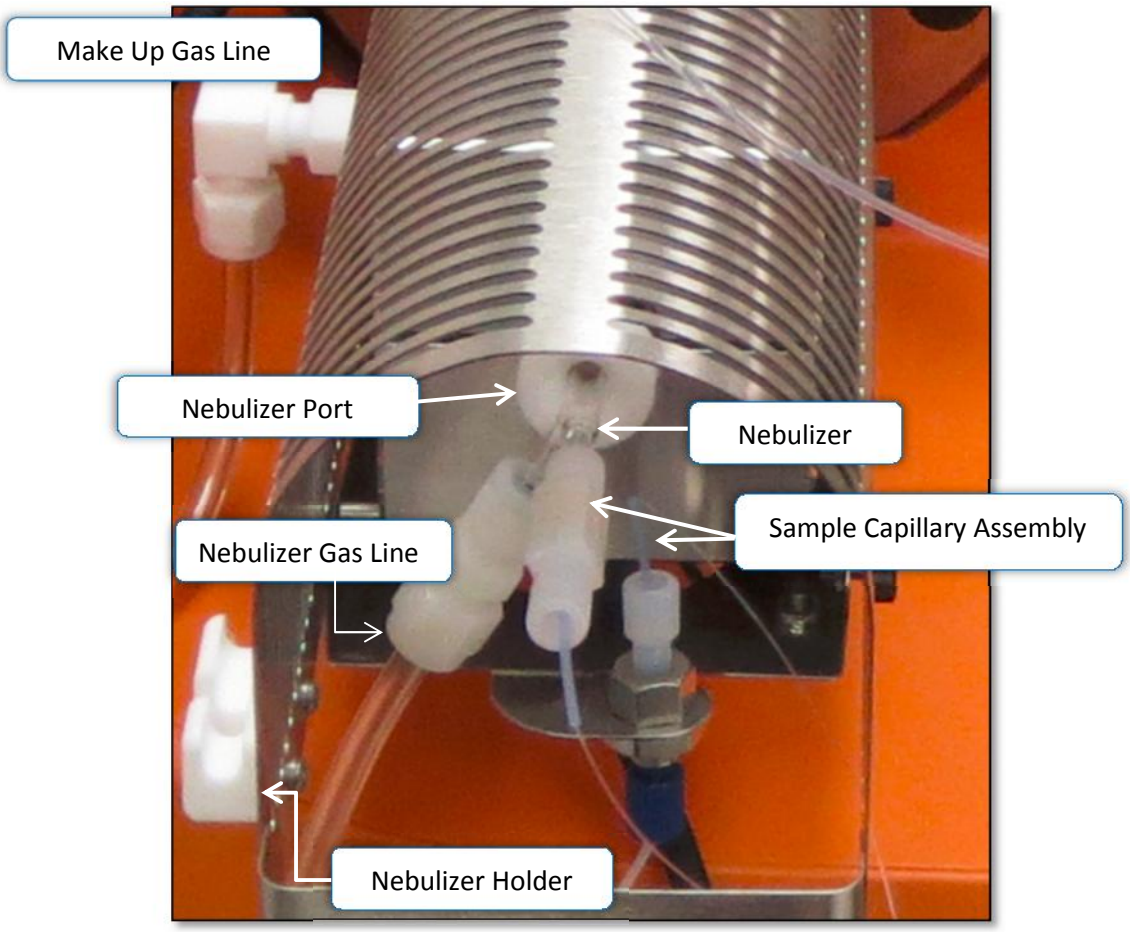


Figure 3.4 Nebulizer and Connections.

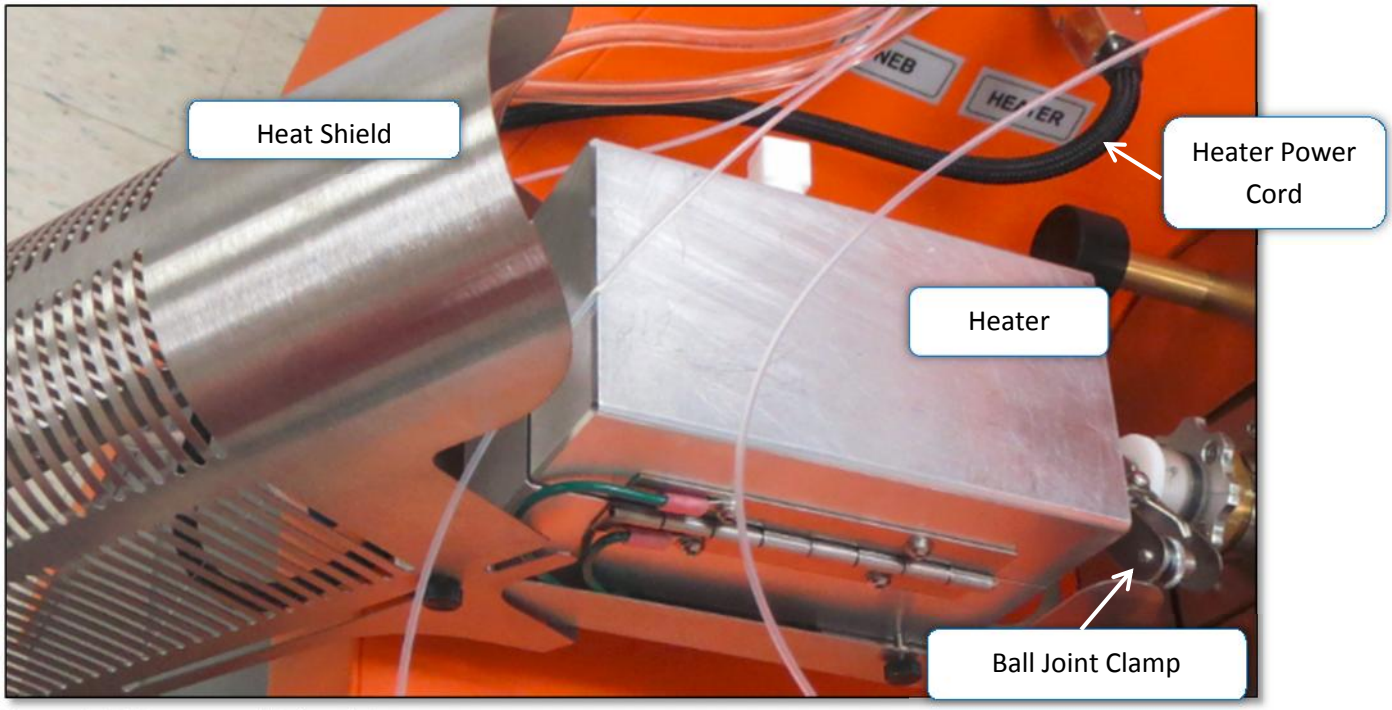


Figure 3.5 Heater and Related Parts.

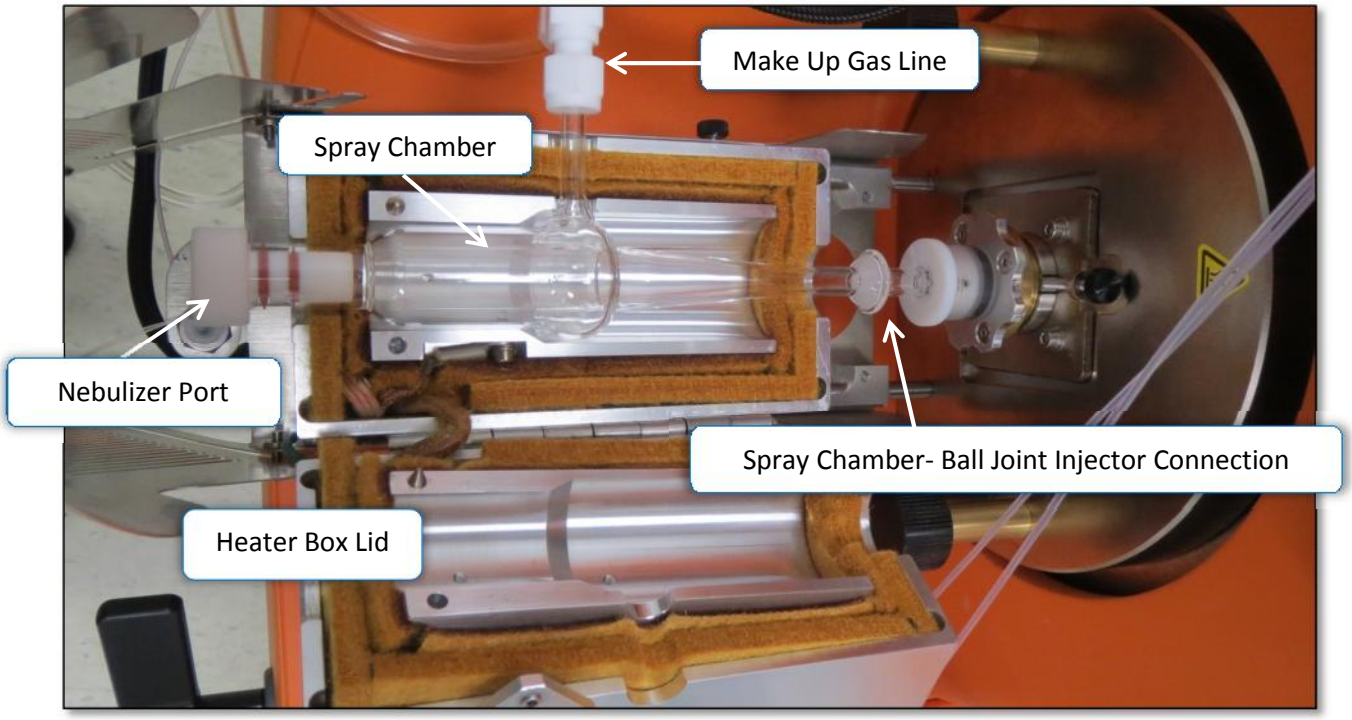


Figure 3.6 Spray Chamber and Connections.

Figure 3.7 Flow Injection Valve and Syringe Pump.

Table 3.1 Flow Injection Valve Configuration.

Port Number	Color Code	Function
1	Blue	Nebulizer Line
2 & 6	Black	Sample Loop 1
3	Brown	Waste (Overflow) Line
4 & 8	Grey	Sample Loop 2
5	White	Upper Syringe Line
7	N/A	Luer Injection Port
N/A	Green	Carrier Reservoir Line

Table 3.2 Alternate Configuration for Flow Injection Valve.

Port Number	Color Code	Function
1	N/A	Luer Injection Port
2 & 6	Black	Sample Loop 1
3	White	Upper Syringe Line
4 & 8	Grey	Sample Loop 2
5	Brown	Waste (Overflow) Line
7	Blue	Nebulizer Line
N/A	Green	Carrier Reservoir Line

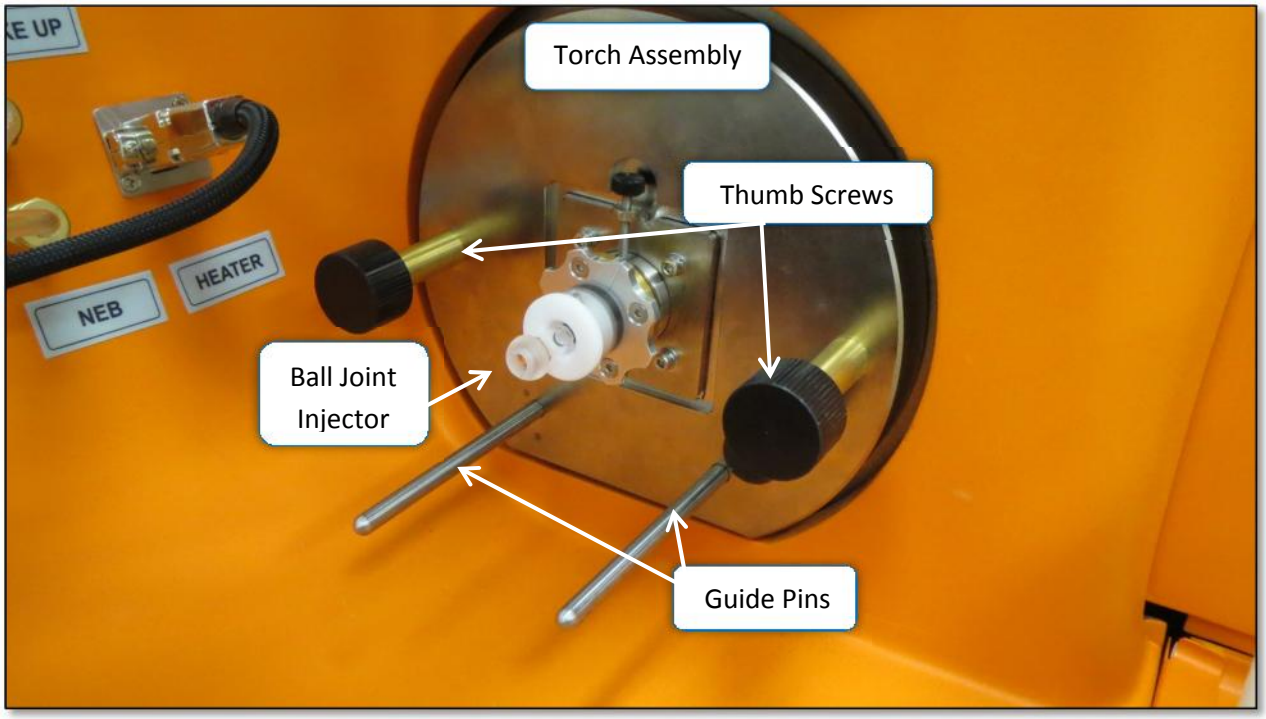


Figure 3.8 Front View of Torch Assembly.

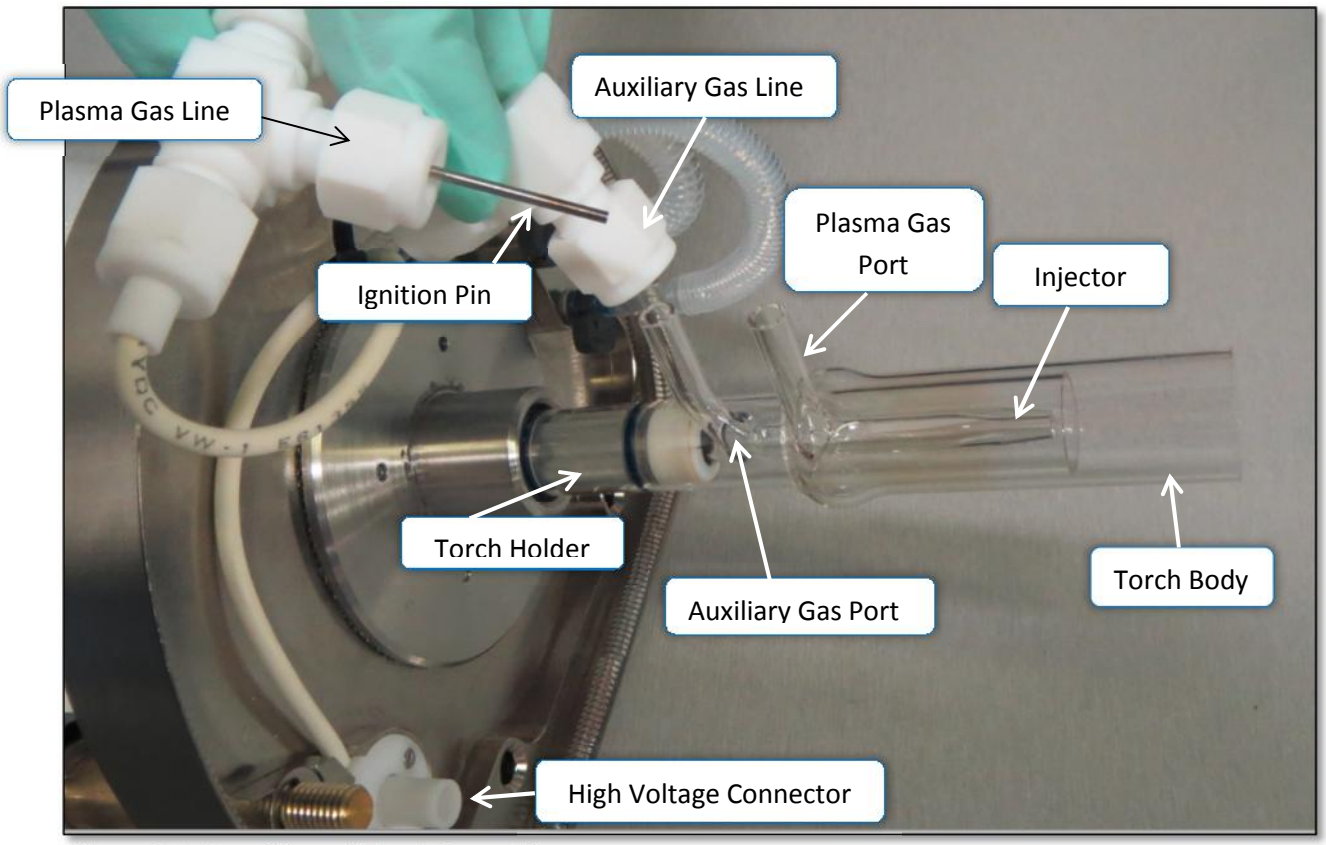


Figure 3.9 Rear View of Torch Assembly.

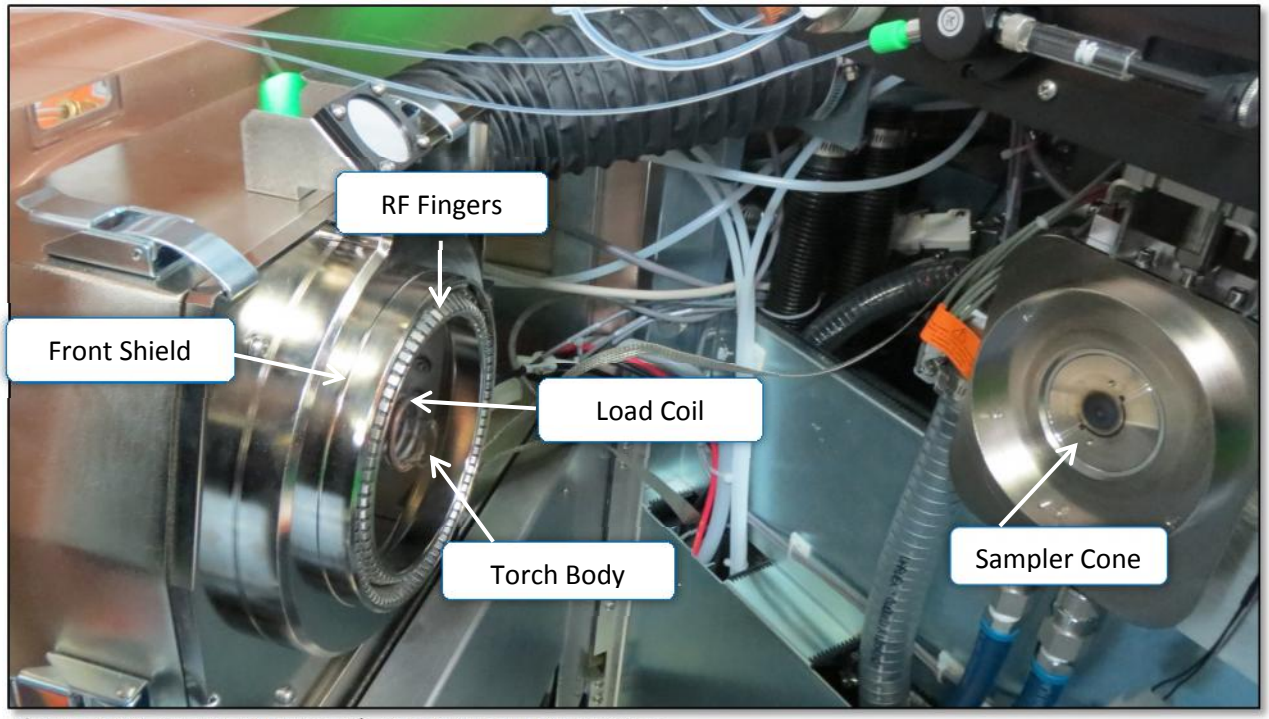


Figure 3.10 Interior View with Front Access Door Open.

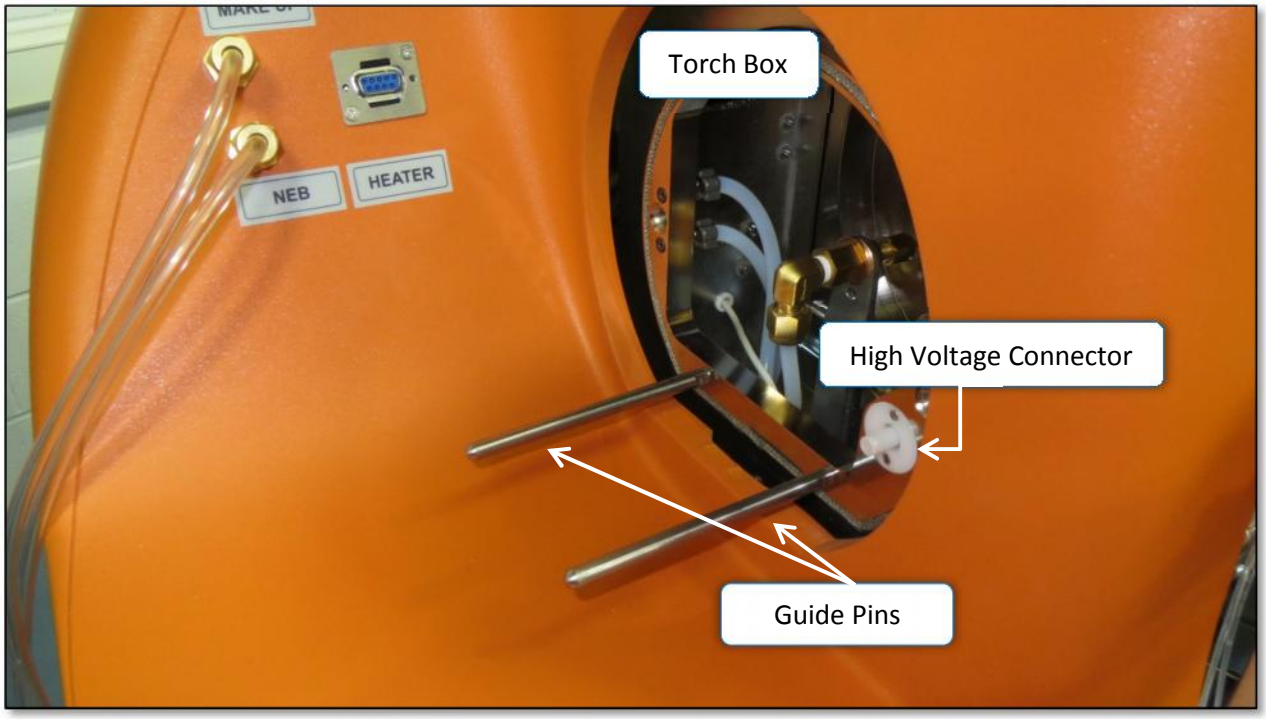


Figure 3.11 Torch Box.

Table 3.3 Other CyTOF 2 Parts.

Parts	Image	Location
Circuit Breakers and Cords		Right Side of Instrument
Digital Readout of Vacuum Gauges, Heater Temperature, Make Up Gas and Nebulizer Gas		Left Side of Instrument
Skimmer/Reducer Cone		Behind Sampler Cone

Table 3.4 CyTOF 2 Glassware.

Part	Image
Nebulizer	
Spray Chamber	
Ball Joint Injector	
Torch	

Chapter 4
Software Interface

Table 4.1 Main Toolbar (Administrator Mode)

Table 4.1

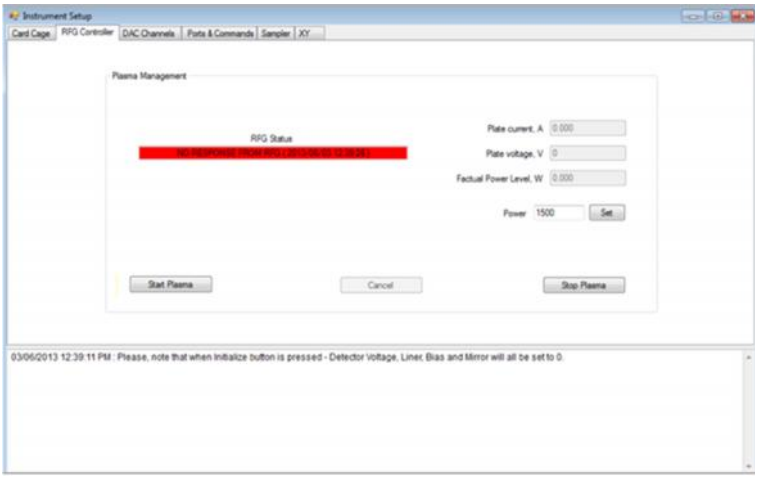
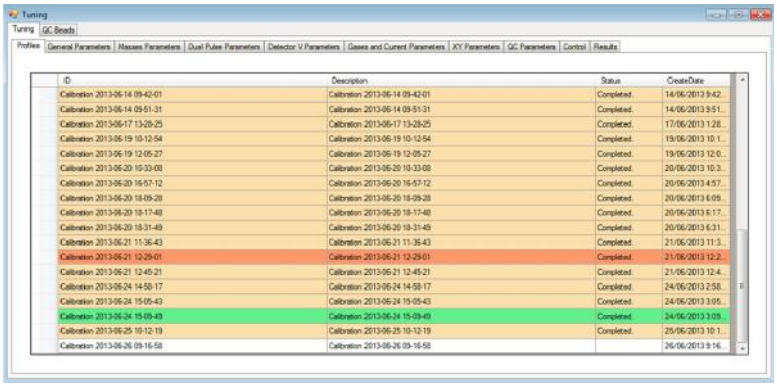
Button	Window	Function
		Start up and shutdown plasma. Access DAC Channels. Perform manual XY alignment.
		Check instrument performance and optimize settings. Run Calibration Beads.

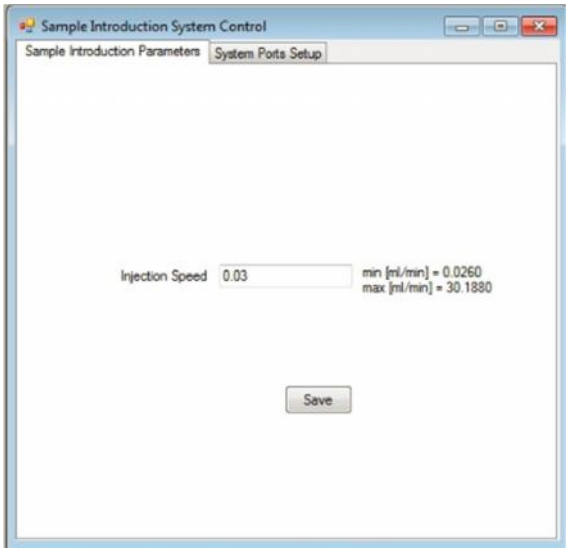
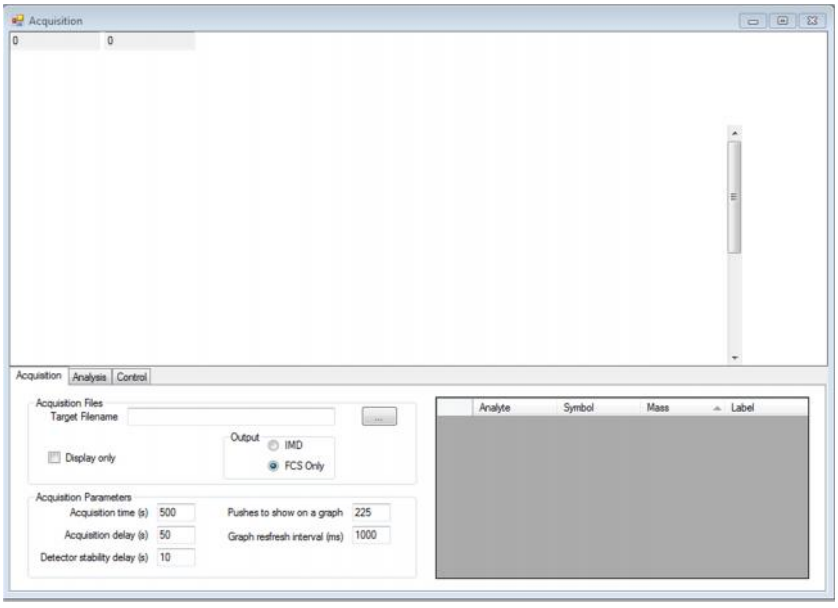
Table 4.1 Button	Window	Function
		Set syringe speed.
		Set parameters for collecting sample data.

Table 4.1

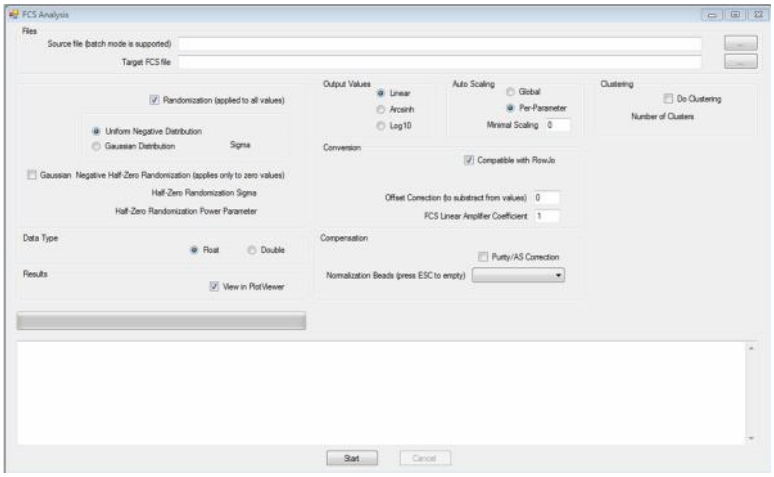
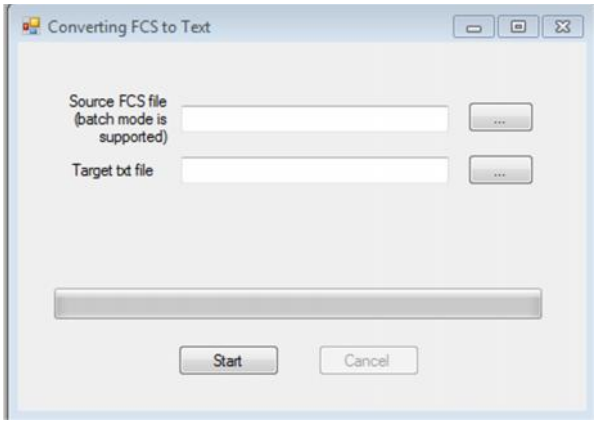
Button	Window	Function
		Set parameters for analysis.
		View data in bivariate plot. Perform clustering of data.
		Convert FCS file to Text format.

Table 4.1

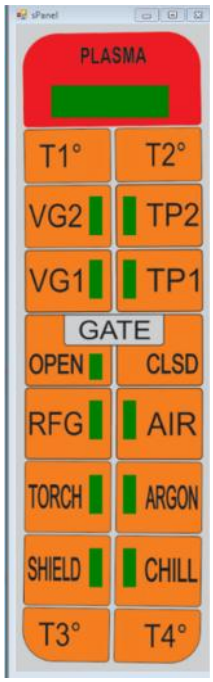
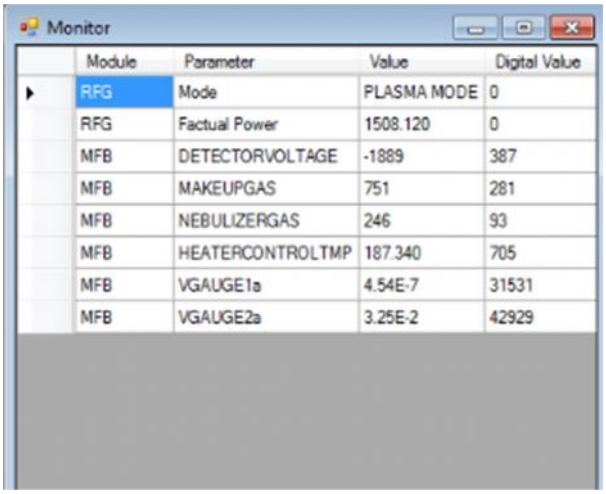
Button	Window	Function
		Software view reflects status of panel on front of CyTOF 2.
		View settings for RFG power, detector voltage, Make Up and Nebulizer Gas, heater temperature and vacuum.
		Launch Cytobank website.

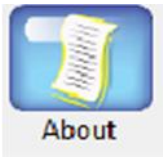
Table 4.1 Button	Window	Function
		Login as User or Service. Check software version.

Table 4.2 Tuning Mode Toolbar

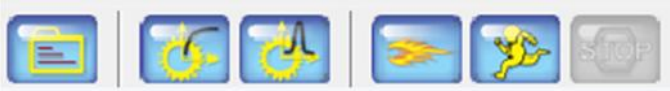


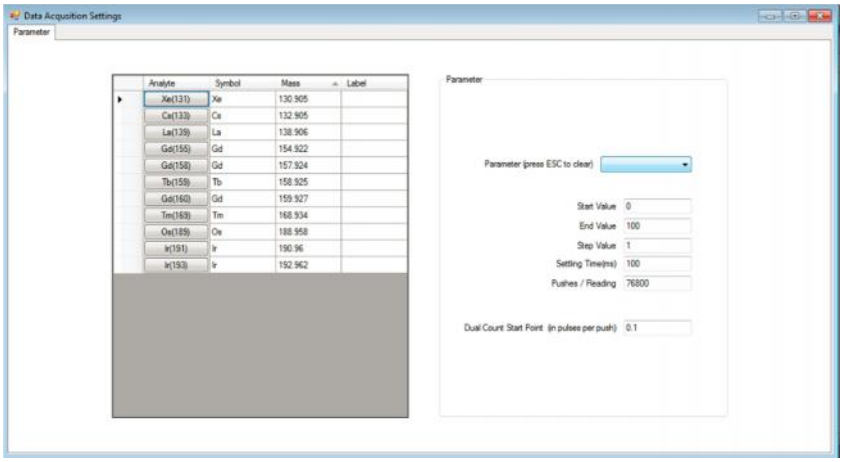
Table 4.2 Button	Window	Function
Data Acquisition Settings 		Choose template and set parameters in Tuning mode.

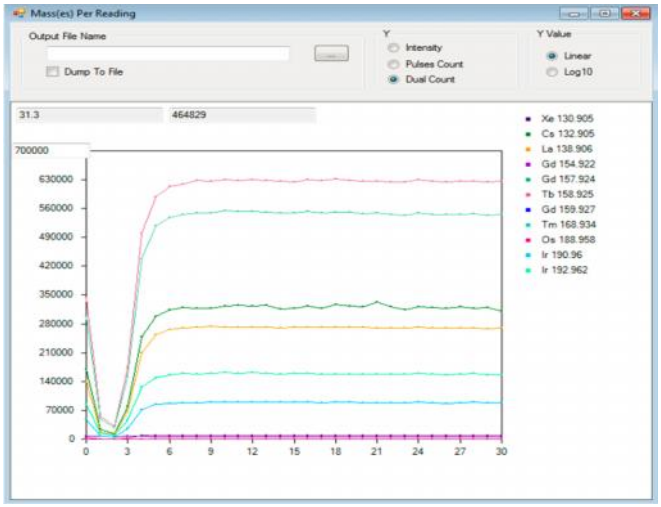
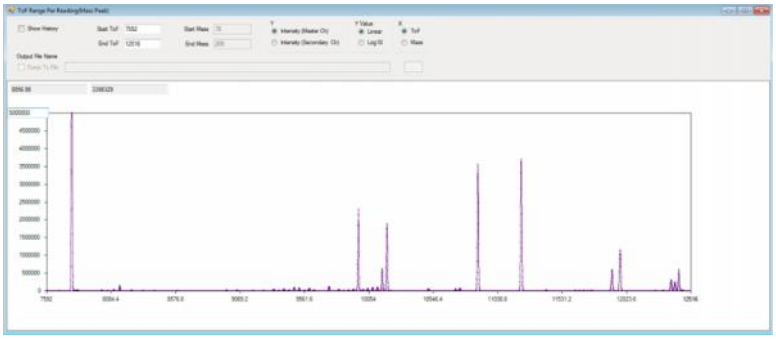
Table 4.2 Button	Window	Function
Mass Per Reading		View intensity, pulse count or dual count of selected isotopes.
Mass Peak (TOF)		View selected Time of Flight (TOF) Range or Mass Range.
Rerun	N/A	Continue to run sample from current sample loop.
Run	N/A	Switch valve to change sample loops.
Stop	N/A	Stop viewing data.

Table 4.3 Syringe Toolbar

Toolbar Item	Function
SP [IDLE] (17:42:25)	Status of syringe pump
Speed [0.045]	Syringe speed (ml/min)
SAMPLE2 (Inj/Tot) [0.520 / 0.520]	Sample Loop In Use Volume of Syringe Injected/Total Volume
	Progress Bar
	Syringe Refresh Button

Chapter 5
CyTOF® 2 Operation

This chapter describes daily operation of the CyTOF® 2 Mass Cytometer, including:

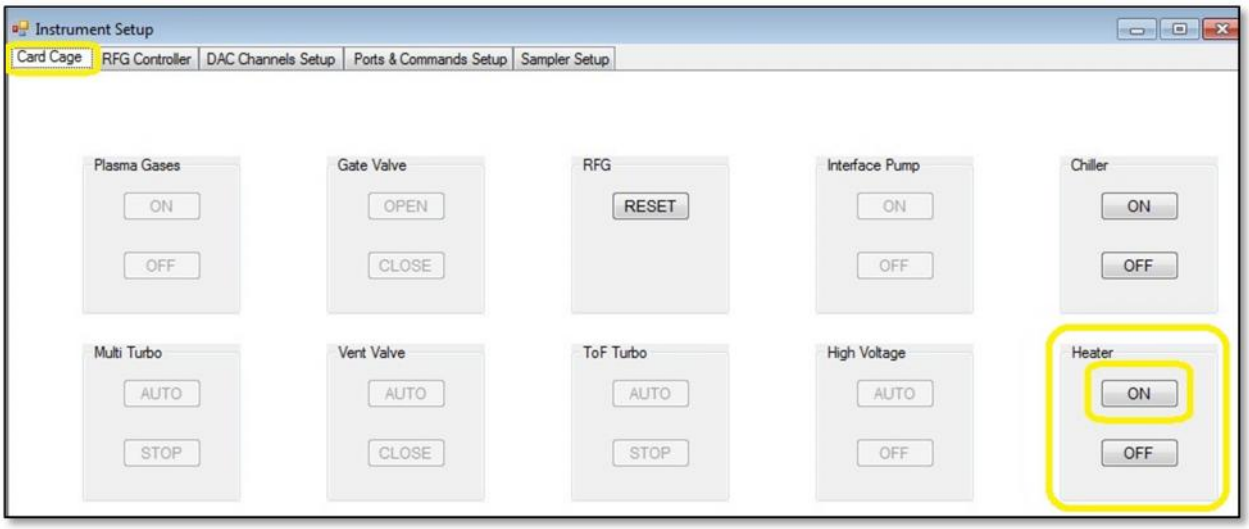
- Preparation and Startup (pp 53-60)
- Overview of the Software Interface and Fluidic System (pp 60-61)
- Daily QC
  - Background Check (p 62)
  - Performance Check (p 63)
  - Auto-Tuning (pp 64-67)
  - Manual Tuning (pp 67-76)
  - Bead Sensitivity Test (p 77)
  - Cleaning after Tuning (p 78)
- Sample Acquisition (pp 79-80)
- Daily Cleaning (p 81)
- Shutdown: Turning off Plasma (p 82)
- Consumables (p 83)

Preparation and Startup

1. Check Status Panel lights. To do so, open the CyTOF Software, and locate the Status Panel (sPanel) within the interface on the left. The panel parameter indicator lights should be lit as in the figure below. If the “ARGON” and “AIR” lights are not green, turn on the argon supply and check that the exhaust (“AIR”) level is correct.

2. Turn on the Heater.
   a. Click

   .
   b. In the **Card Cage tab of** the Instrument Setup page, click on **Heater**> **On**.
   c. The Heater module will take ~ 20 minutes to reach 195 to 200 °C.

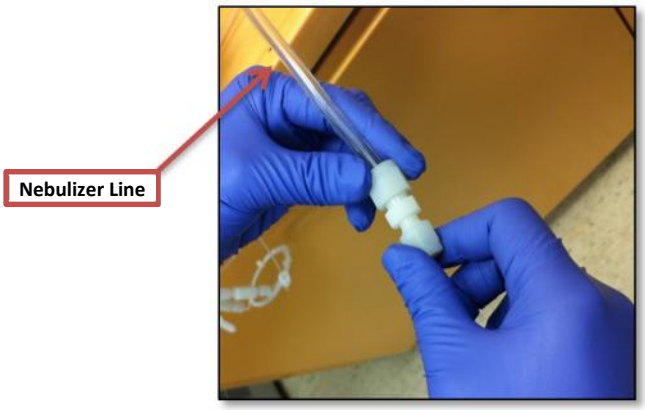


3. While the Heater is warming up, connect the Nebulizer following the steps below.

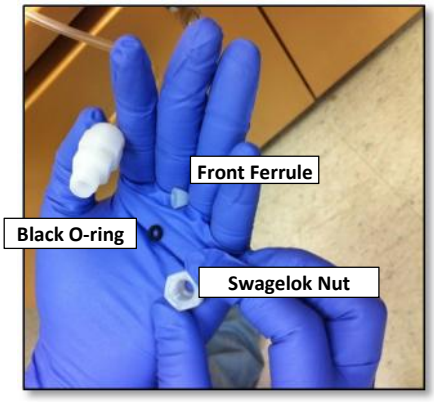
Assemble and Install the Nebulizer

(Important: wear gloves to prevent finger oil contamination on the nebulizer glass)

1. Unscrew Swagelok Nut from the connector at the end of the Nebulizer Gas line.

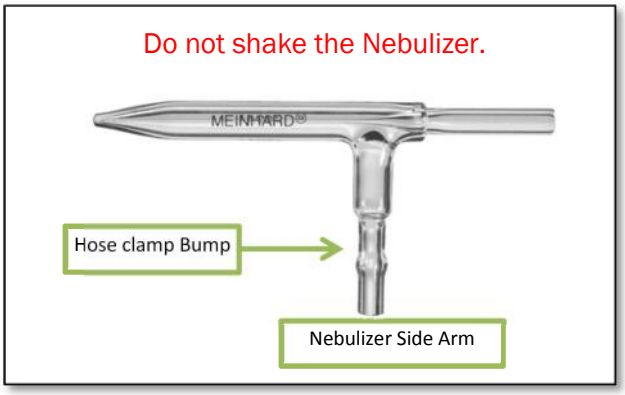


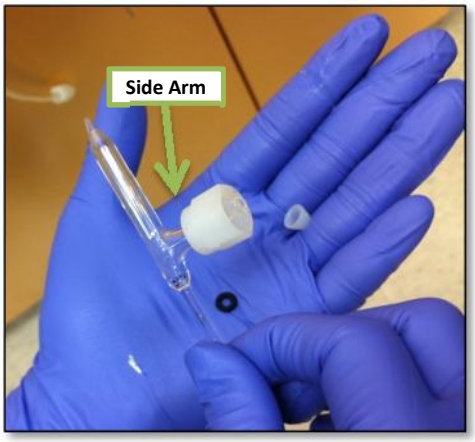
2. Remove the Front Ferrule and the black O-ring.



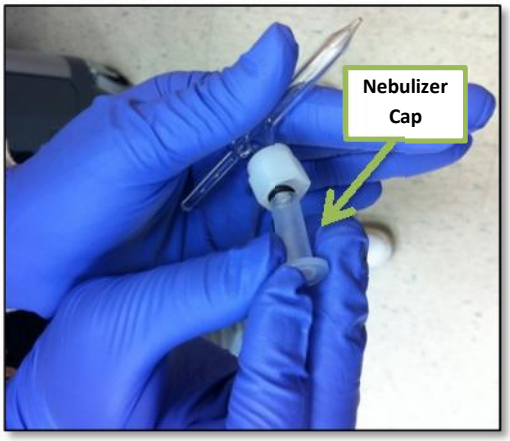
3. Remove the clean Nebulizer from the Nebulizer soaking container and dry the surface with Kimwipe (Do not touch tip of Nebulizer with Kimwipe.). Excess water inside the Nebulizer should also be removed.

4. Put the side arm of the Nebulizer through the Swagelok Nut.

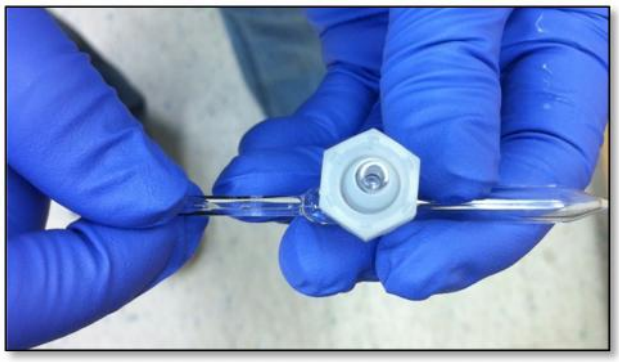




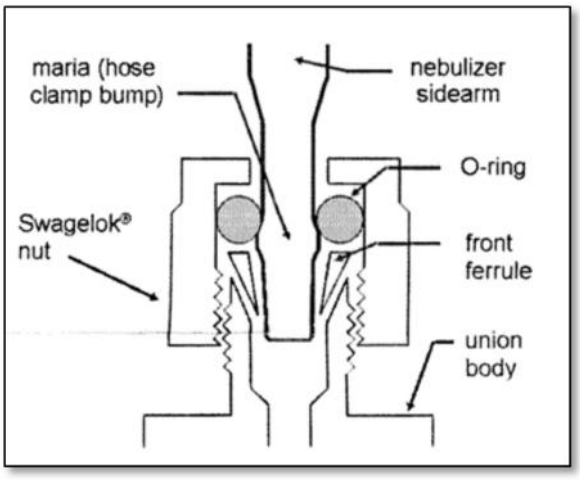
5. Push the O-ring onto the side arm with a tool, such as the Nebulizer cap, pushing it over the hose clamp bump on the Nebulizer side arm.



6. Place the ferrule over the O-ring with smaller orifice facing away from the Nebulizer.

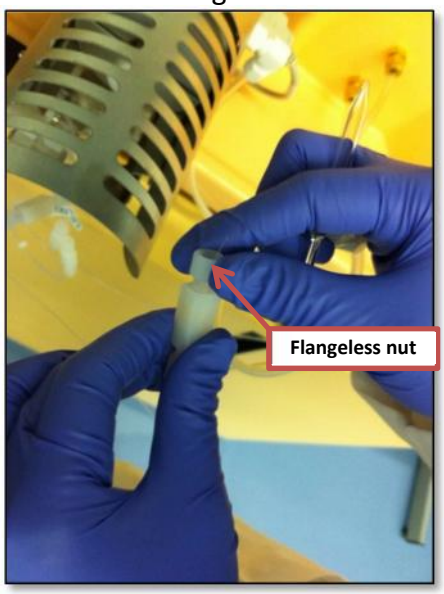


7. Screw the nut of the union back together.



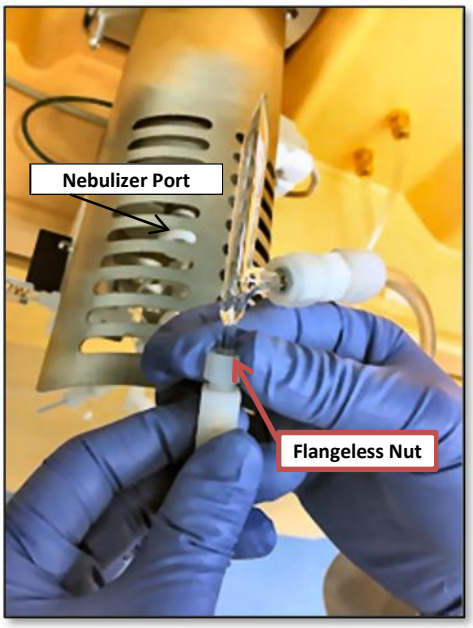
Schematic of the Nebulizer side arm

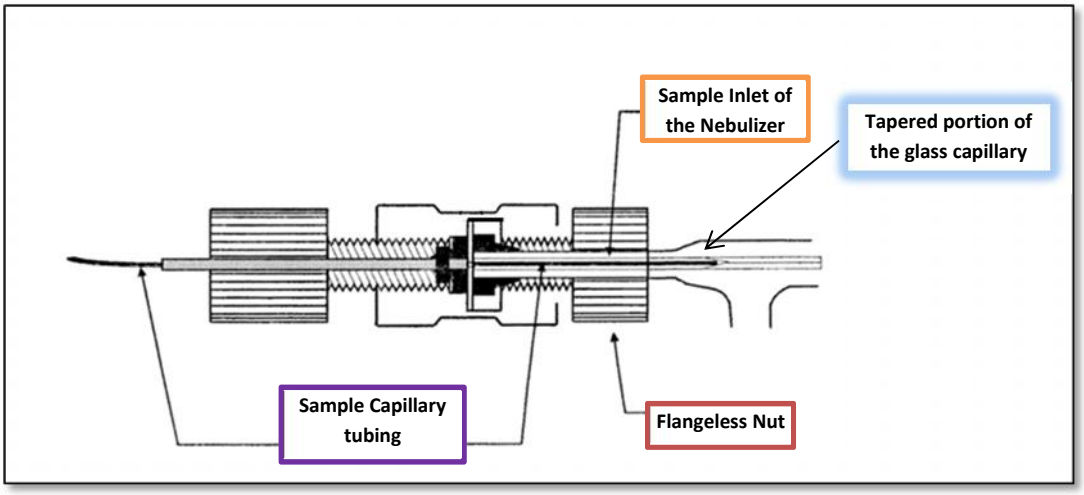
8. Connect Sample Capillary tube to Nebulizer:
    a. Loosen the Flangeless Nut on the connector of the Sample Capillary.



b. Insert the Sample Capillary tubing into the sample inlet end of the Nebulizer and push up to tapered portion of the glass capillary inside the Nebulizer.

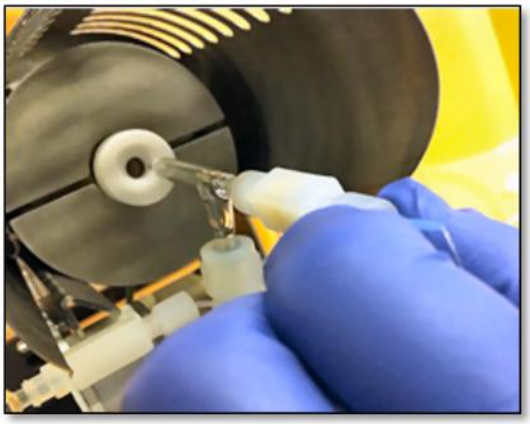
c. Tighten the Flangeless Nut.

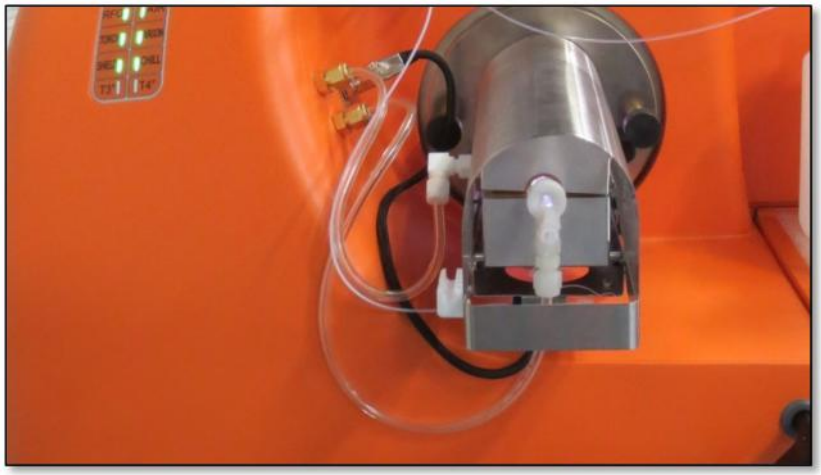




Schematic of sample capillary connected to nebulizer

d. Insert the Nebulizer into the Nebulizer Port attached to spray chamber until it reaches hard stop point.



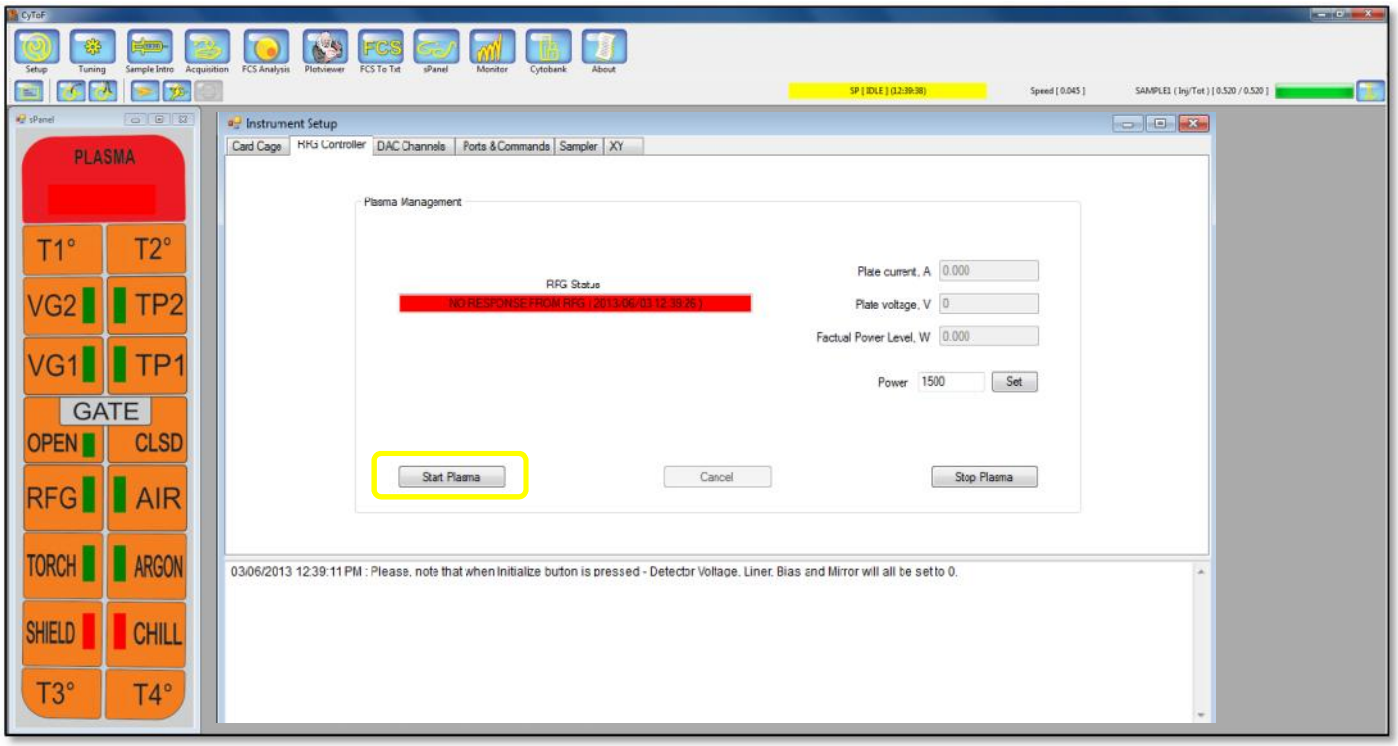


Optional: **Removing Excess Water from the Nebulizer**

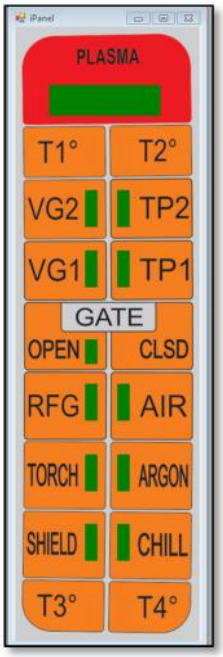
1. Remove Nebulizer from Nebulizer Port.
2. In Setup>DAC Channels, find Nebulizer Gas and note setting.
3. Click Set Actual Current Value. This will start the flow of Nebulizer Gas.
4. When water in Nebulizer is gone, go back to Nebulizer Gas in DAC Channels and set value to 0 and click **Set Actual Current Value**. This will turn off the Nebulizer Gas flow.
5. Set the Nebulizer Gas back to the original setting and Click Save.
6. Insert the Nebulizer in the Nebulizer Port until it reaches a hard stop.

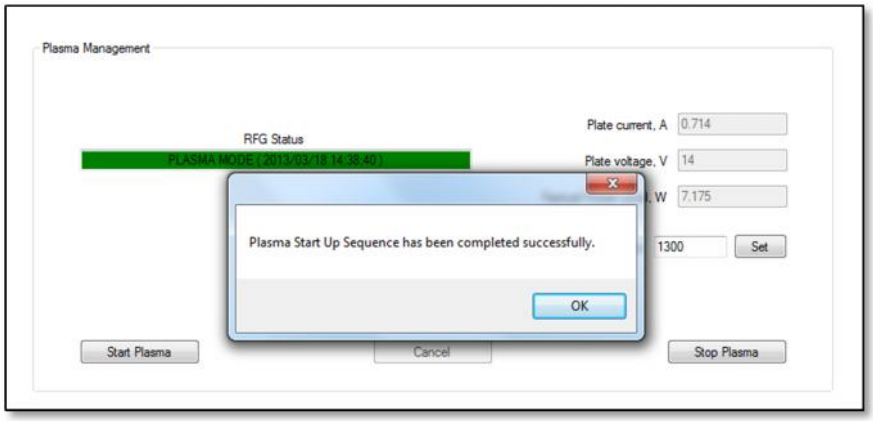
Plasma Start

1. Check the Status Panel lights and ensure that the Argon light is green.
2. Fill Carrier reservoir and empty Waste.
3. In **Instrument setup** > **RFG Controller**, click on **Start Plasma**



4. When plasma starts, the software will give you the following message and the status panel (left) is updated (see following graphic):



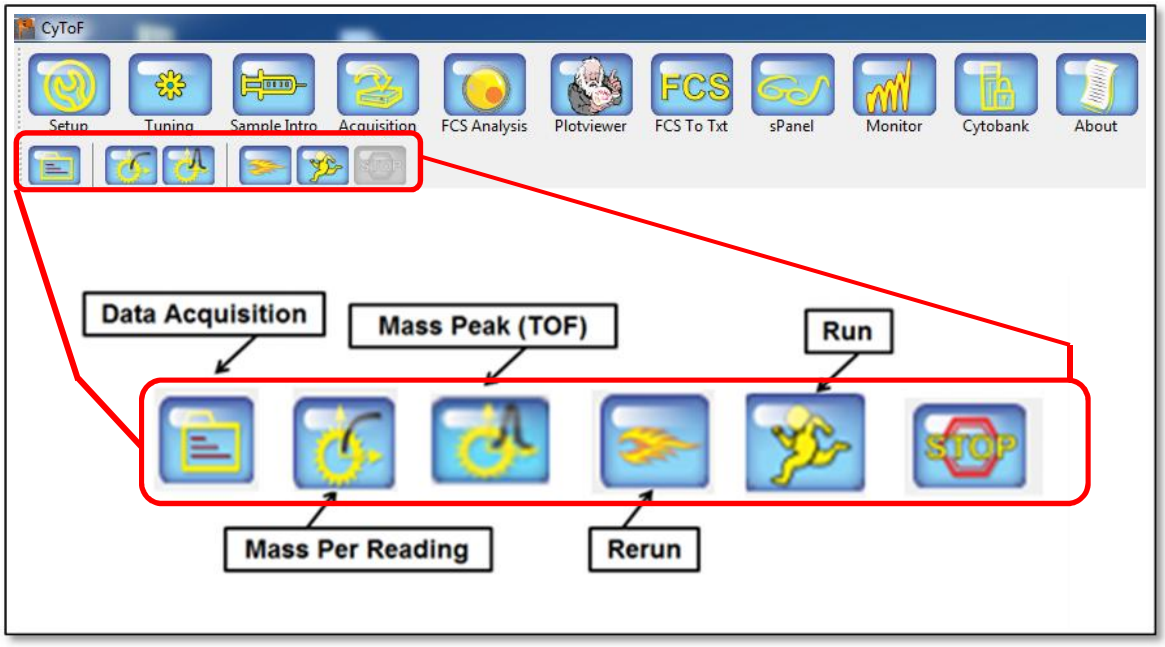


5. Click "**OK**" and allow plasma to warm up for 15-30 minutes.

Overview of the Software Interface and Fluidic System

This section provides a brief overview of the software interface and fluidic system.

Software Interface

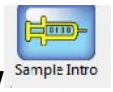


Fluidic System

The CyTOF 2 utilizes a syringe pump connected to a dual-loop system for sample introduction. Two sample loops (1 and 2) are connected to a single sample line through a flow injection valve. Once plasma has been lit, the syringe pump continuously pushes carrier fluid (DIW) into the active sample loop, as indicated by the software (see red box in figure below). When a new sample is loaded, it will fill the idle loop and be held there until the operator clicks either the "Run" or "Preview" button. When either of these buttons is clicked, the valve switches and carrier fluid is pushed through the previously idle loop, and data acquisition of the newly-loaded sample begins. The previously active loop is then idle and available for loading of another sample. Selecting "Re-run" or "Re-preview" will not cause valve switching and so sample acquisition will continue to be from the currently active loop..

Users can check what loop is in use on the upper right syringe pump status bar as shown below:

For optimal signal intensity and resolution, the Syringe Pump speed is set at **45uL/min** (0.045 mL/min) and this defines the sample flow rate. The maximum flow rate at which plasma can be sustained is 60uL/min (0.060 mL/min).

The syringe pump flow rate can be changed in the **Sample Intro window** 

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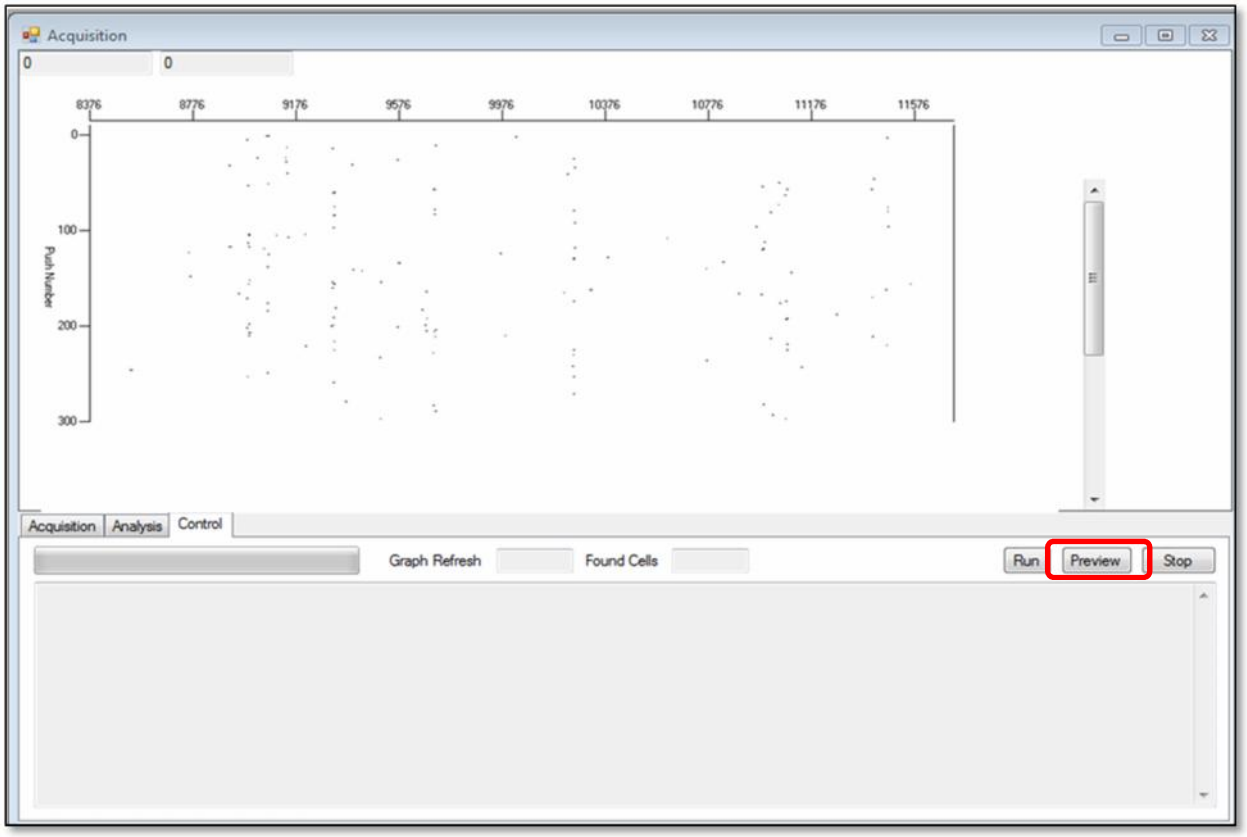
Daily QC

The CyTOF2 should be tuned every day for optimal performance and data quality. Tuning can be performed automatically or manually.

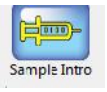
Check Background

1. Open the **Acquisition** window

.
2. Click on the **Control** tab.
3. Click **Preview** to view background signal and ensure that the sample introduction system is clean and ready for Tuning.



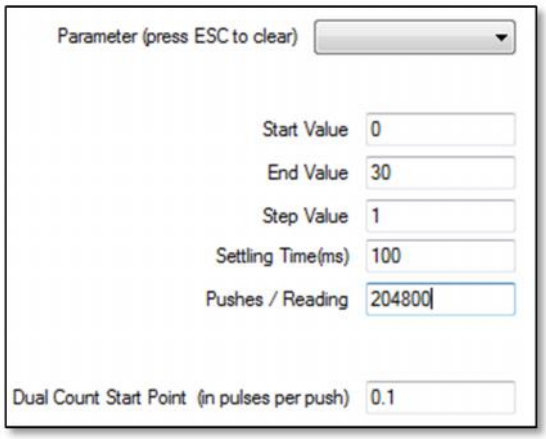
Check Performance before Tuning

1. Ensure that the Syringe Pump speed is set to 0.045 mL/min in **Sample Intro** 

, or by checking the upper right portion of the software interface.

2. Inject 500uL of Tuning Solution into a Sample Loop.

3. In the **Data Acquisition Settings** window , set up the data acquisition parameters as follows:


Parameter (press ESC to clear)
Start Value 0
End Value 30
Step Value 1
Settling Time(ms) 100
Pushes / Reading 204800
Dual Count Start Point (in pulses per push) 0.1

Parameter is “Reading” by default if empty. Set Pushes/Reading to 204,800.

(Note: Since there are 76,800 pushes per second, 204,800 pushes equals 2.67 seconds per reading.)

4. Open the **Masses per Reading** window and select Dual counts for the Y axis and set max pulse counts to an appropriate value for your instrument.
5. Click “Run”
6. Wait until the signal stabilizes and observe ^{159}Tb and Mass 155 (^{155}Gd) Dual count values.
   a. If ^{159}Tb dual count levels are comparable to the levels in a well performing operating session (>400K with 204800 pushes per reading), AND
   b. ^{155}Gd/^{159}Tb ratio is below 3% and comparable to the level from previous days with good performance, begin **Auto-Tuning** or **Manual Tuning**.

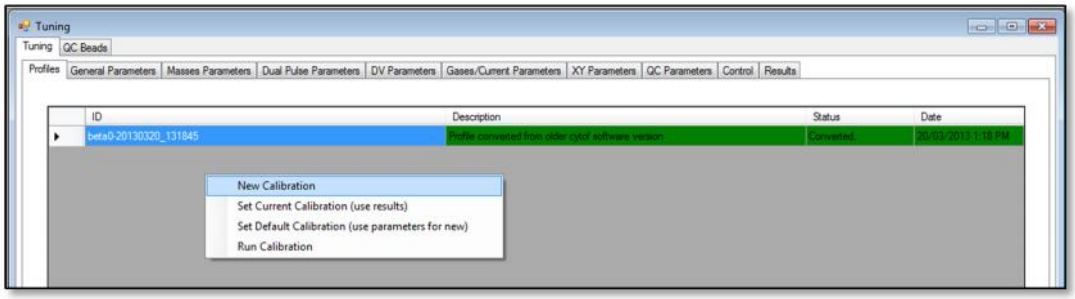
c. If the signals are below specification, adjust XY alignment manually (see **Manual Tuning > XY Alignment** section below) and begin Auto-Tuning or Manual Tuning.

Auto-Tuning

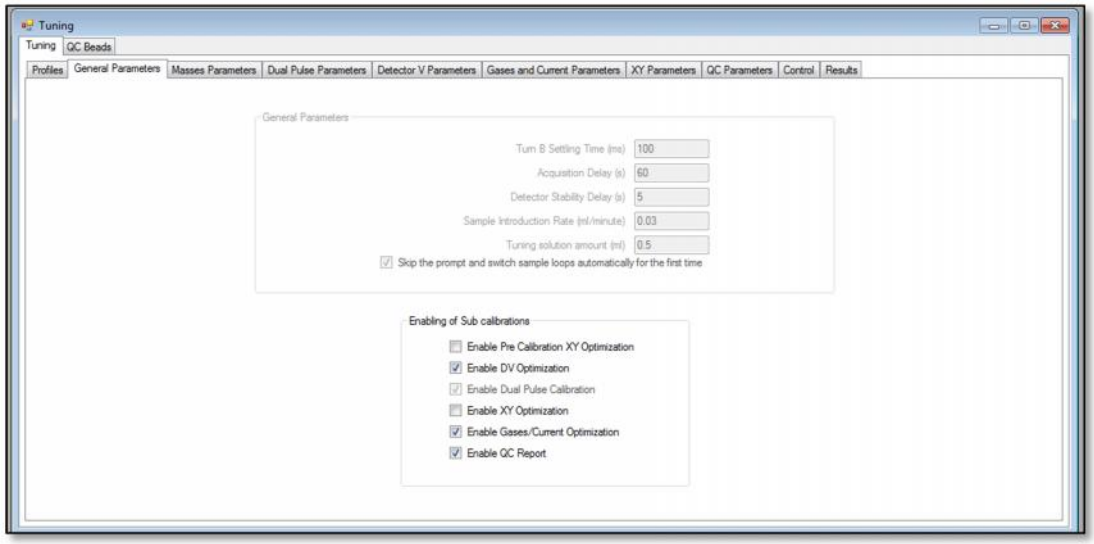
Note: If any of the settings are different than described below, Administrators may need to access the service mode temporarily to change these settings. If applicable, see note for "***Service Access***" in the following sections.

1. Click on Tuning.

2. Select "Tuning" Tab for Auto-Tuning.
3. In the Profiles Tab, right-click and select "New Calibration".

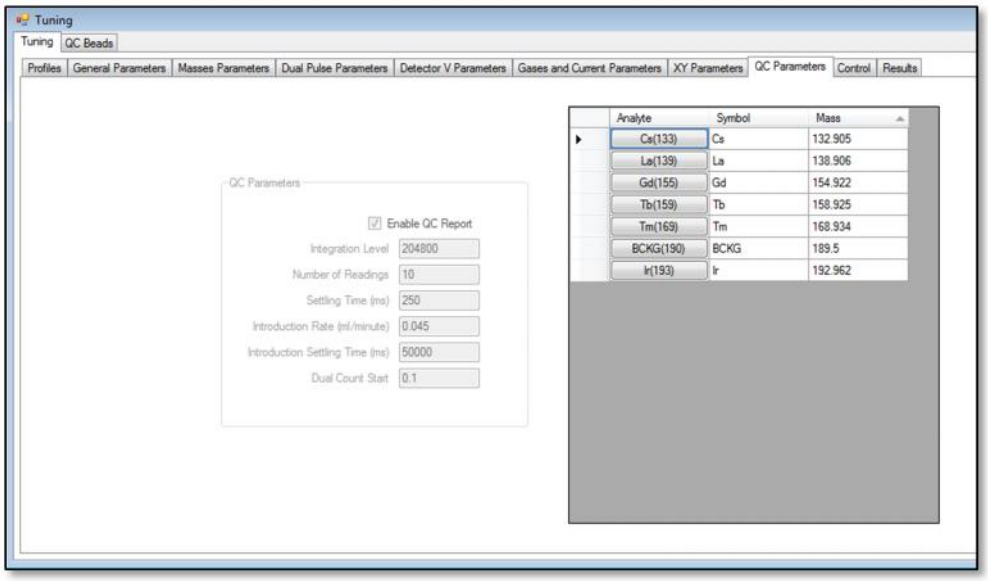


4. Under General Parameters, ensure that all the desired tuning parameters are checked with proper delay timings. Perform Auto-tuning with **DV Optimization**, **Dual Pulse Calibration**, **Gases/Current Optimization** and **QC report** enabled in General Parameters. **Mass calibration** and **Mass Resolution** are automatically performed and calculated whenever Auto-Tuning is performed.

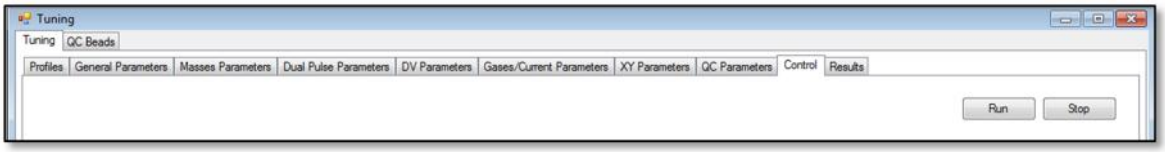


Service Access Notes :

1. Note that introduction rate is pre-set to 0.03 mL/min in General Parameters. Do not change this setting.
2. Under QC parameters, ensure that all of the relevant tuning analytes are selected with the correct parameters as shown in the following figures.
   a. If necessary, right click or hit F4 on the Keyboard to pull up the Periodic Table to select any additional analytes.
   b. Ensure that the integration level is set to 204,800 (pushes) and introduction rate is 0.045 (mL/min).

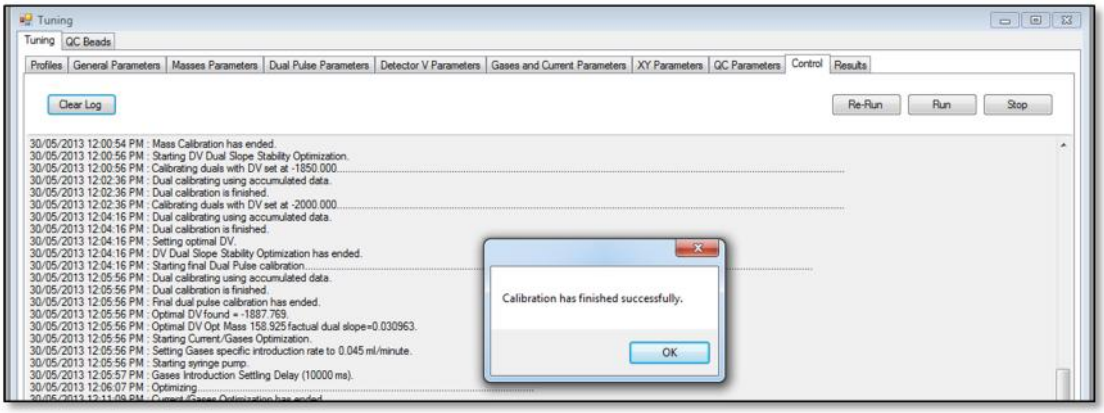


5. Inject 500uL of Tuning Solution, and click “Run” under the control tab

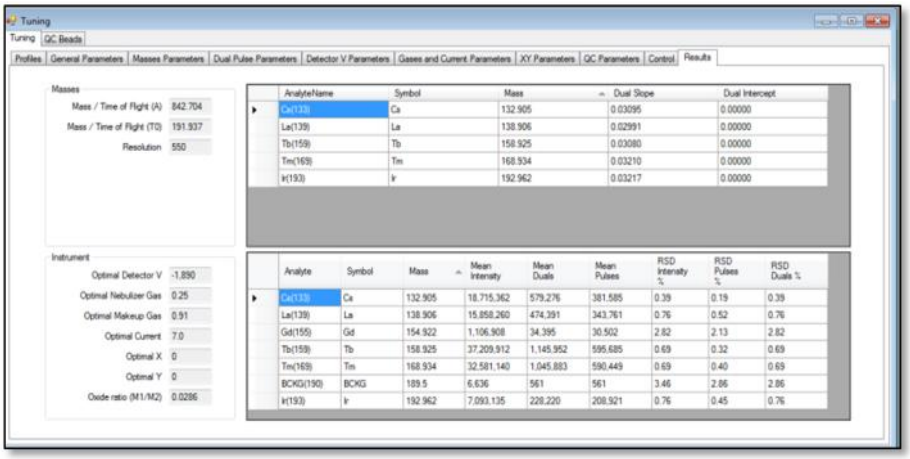


6. After clicking “Run”, the flow injection valve will switch and the other loop will be available for a new sample.
7. Inject another 500uL into the injection port. Once the first loop is finished, the second loop of tuning solution will automatically be acquired for the Auto-Tuning process to continue.
8. A progress log will appear in the control screen as below.

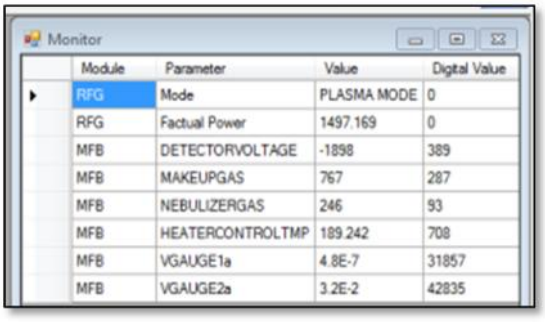
9. When calibration finishes successfully, click OK.


Tuning
Tuning
QC Beads
Profiles
General Parameters
Masses Parameters
Dual Pulse Parameters
Detector V Parameters
Gases and Current Parameters
XY Parameters
QC Parameters
Control
Results
Clear Log
Re-Run
Run
Stop
30/05/2013 12:00:54 PM : Mass Calibration has ended.
30/05/2013 12:00:56 PM : Starting DV Dual Slope Stability Optimization.
30/05/2013 12:00:56 PM : Calibrating duals with DV set at -1850.000.
30/05/2013 12:02:36 PM : Dual calibrating using accumulated data.
30/05/2013 12:02:36 PM : Dual calibration is finished.
30/05/2013 12:02:36 PM : Calibrating duals with DV set at -2000.000.
30/05/2013 12:04:16 PM : Dual calibrating using accumulated data.
30/05/2013 12:04:16 PM : Dual calibration is finished.
30/05/2013 12:04:16 PM : Setting optimal DV.
30/05/2013 12:04:16 PM : DV Dual Slope Stability Optimization has ended.
30/05/2013 12:04:16 PM : Starting final Dual Pulse calibration.
30/05/2013 12:05:56 PM : Dual calibrating using accumulated data.
30/05/2013 12:05:56 PM : Dual calibration is finished.
30/05/2013 12:05:56 PM : Final dual pulse calibration has ended.
30/05/2013 12:05:56 PM : Optimal DV found = -1887.769.
30/05/2013 12:05:56 PM : Optimal DV Opt Mass 158.925 factual dual slope=0.030963.
30/05/2013 12:05:56 PM : Starting Current/Gases Optimization.
30/05/2013 12:05:56 PM : Setting Gases specific introduction rate to 0.045 ml/minute.
30/05/2013 12:05:56 PM : Starting syringe pump.
30/05/2013 12:05:57 PM : Gases Introduction Settling Delay (10000 ms).
30/05/2013 12:06:07 PM : Optimizing.
Calibration has finished successfully.
OK

10. In the Results Tab, ensure the ^{159}Tb mean dual value is at least 400K and that the RSD is less than 3% (Note: RSD is relative standard deviation and is equivalent to CV). If not, perform XY alignment manually (see Manual Tuning section) and then repeat the Auto-Tuning process from step 1.

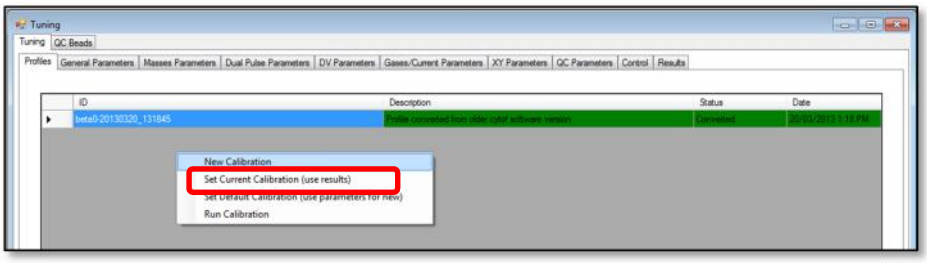

Tuning
Tuning
QC Beads
Profiles
General Parameters
Masses Parameters
Dual Pulse Parameters
Detector V Parameters
Gases and Current Parameters
XY Parameters
QC Parameters
Control
Results
Masses
Mass / Time of Flight (A) 842.704
Mass / Time of Flight (TO) 191.937
Resolution 550
Instrument
Optimal Detector V -1.890
Optimal Nebulizer Gas 0.25
Optimal Makeup Gas 0.91
Optimal Current 7.0
Optimal X 0
Optimal Y 0
Oxide ratio (M1/M2) 0.0286
AnalyteName Symbol Mass Dual Slope Dual Intercept
Ce(133) Ce 132.905 0.03095 0.00000
La(139) La 138.906 0.02991 0.00000
Tb(159) Tb 158.925 0.03080 0.00000
Tm(169) Tm 168.934 0.03210 0.00000
Ir(193) Ir 192.962 0.03217 0.00000
Analyte Symbol Mass Mean Intensity Mean Duals Mean Pulses RSD Intensity % RSD Pulses % RSD Duals %
Ce(133) Ce 132.905 18,715,362 579,276 381,585 0.39 0.19 0.39
La(139) La 138.906 15,858,260 474,391 343,761 0.76 0.52 0.76
Gd(155) Gd 154.922 1,106,908 34,395 30,502 2.82 2.13 2.82
Tb(159) Tb 158.925 37,209,912 1,145,952 595,685 0.69 0.32 0.69
Tm(169) Tm 168.934 32,581,140 1,045,883 590,449 0.69 0.40 0.69
BCKG(190) BCKG 189.5 6,636 561 561 3.46 2.86 2.86
Ir(193) Ir 192.962 7,093,135 228,220 208,921 0.76 0.45 0.76

11. The profile will be automatically applied and can be verified in the Monitor Window:


Monitor
Module Parameter Value Digital Value
RFG Mode PLASMA MODE 0
RFG Factual Power 1497.169 0
MFB DETECTORVOLTAGE -1898 389
MFB MAKEUPGAS 767 287
MFB NEBULIZERGAS 246 93
MFB HEATERCONTROLTMP 189.242 708
MFB VGAUGE1a 4.8E-7 31857
MFB VGAUGE2a 3.2E-2 42835

Note: Values in the Monitor window are actual readings. Some of these may not match the Set values exactly (the optimal values displayed).

12. If settings are changed, they can be restored by selecting the Tuning Profile and right-clicking to choose “Set Current Calibration (use results).”



This will set any values optimized during the calibration run (i.e. Dual Slope, Detector voltage, Gas Settings, etc.).

13. Record pertinent values from the Results tab, including:

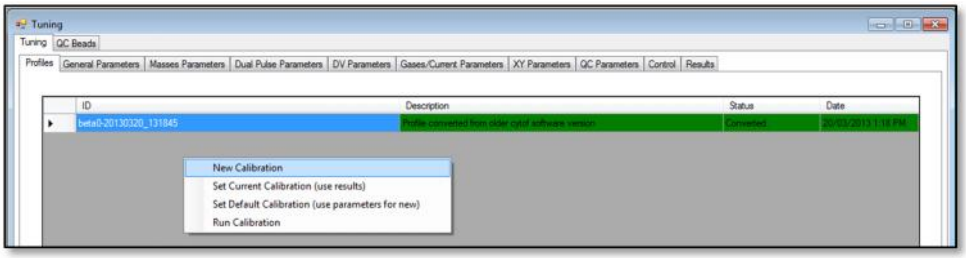
    a. Resolution
    b. Dual slope values for Cs and Tm
    c. Mean Dual Count Tb value
    d. RSD (Dual) values for: Tb, Cs, La, Tm, Ir
    e. Mean 155Gd Dual counts
    f. DAC Channels settings: Detector Voltage, Nebulizer Gas, Makeup Gas, Current

Manual Tuning

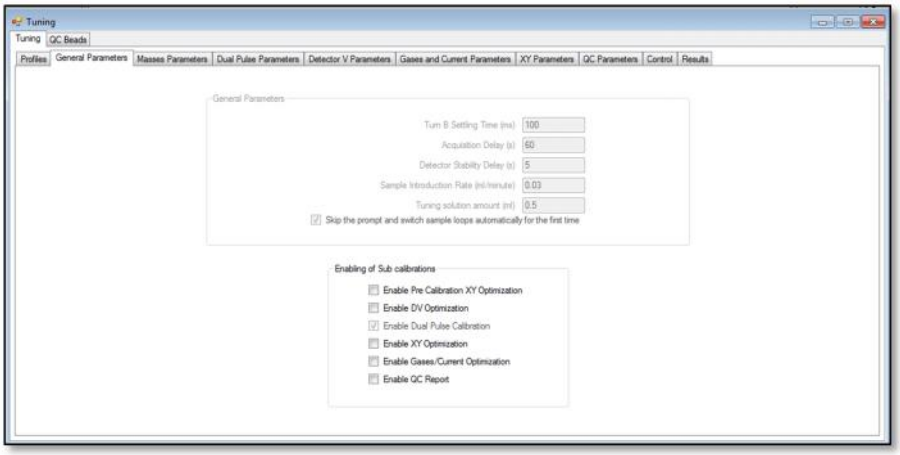
If Auto-Tuning is unsuccessful due to reasons that cannot be resolved by changing appropriate parameters, proceed to perform Manual Tuning as described in the following steps.

Mass Calibration

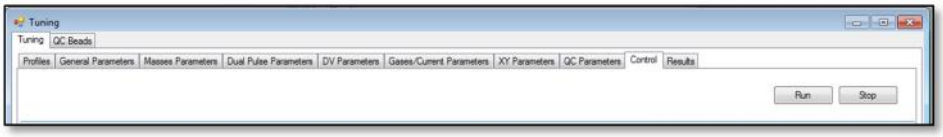
1. Inject Tuning Solution.
2. Select Tuning > Profile, right-click and select New Calibration.



3. In the General Parameters tab, de-select all tuning parameters except for Dual Calibration (which is selected by default).



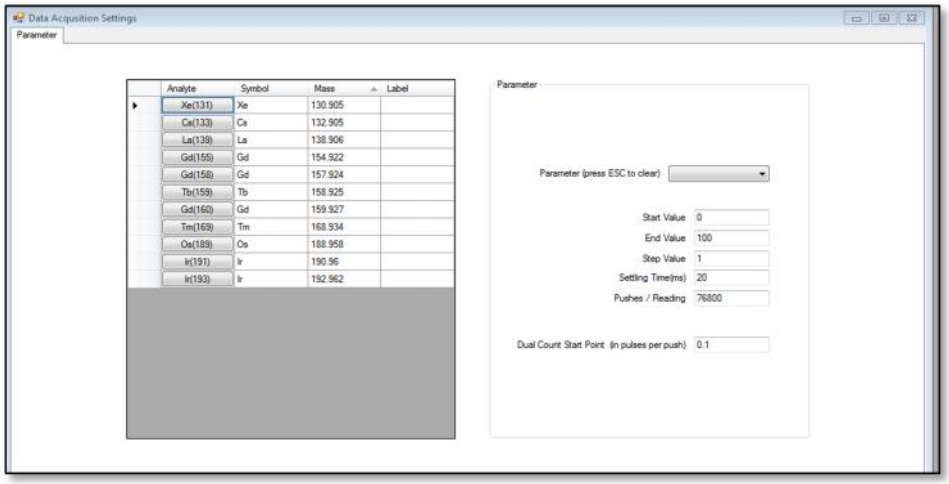
4. Select Control tab, click “Run”.
   (Note: Running with no parameters selected will activate only Mass Calibration and Dual Pulse Calibration.)



XY Alignment

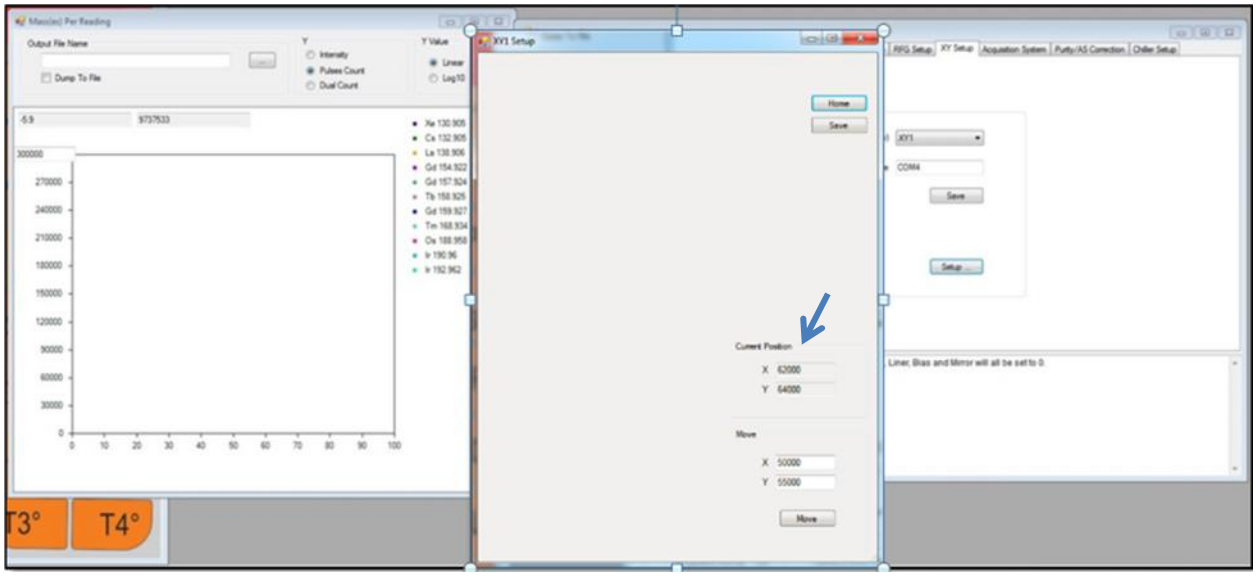
1. If needed, inject another 500uL of Tuning Solution.

2. Set up the data acquisition parameters in the **Data Acquisition Settings** window .
   a. Parameter is “Reading” by default if empty.
   b. Set Pushes/Reading to 76,800 to allow 1 reading per second.
   c. Enter an End Value long enough for the alignment to complete. An End Value of 200 (acquisition time of 100 seconds) is usually sufficient.



3. Open the **Masses per Reading** window
4. Select pulse counts for the Y axis and set max pulse counts to a value which is approximately one third to one half of the Dual Counts value for the system.
5. Click “Re-Run” .
6. Select Setup

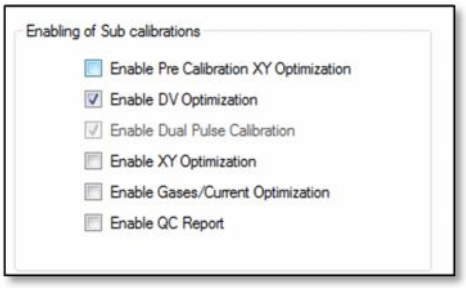
> XY setup > Setup
7. Align the window such that both windows are visible.



8. Note the Current Position for X and Y before making any changes (see blue arrow in figure above.)
9. While observing the pulse count signal in the Masses per Reading graph, change X value by steps of 3000 until signal is at its highest.
10. Adjust by smaller steps if necessary.
11. Repeat for the Y value.

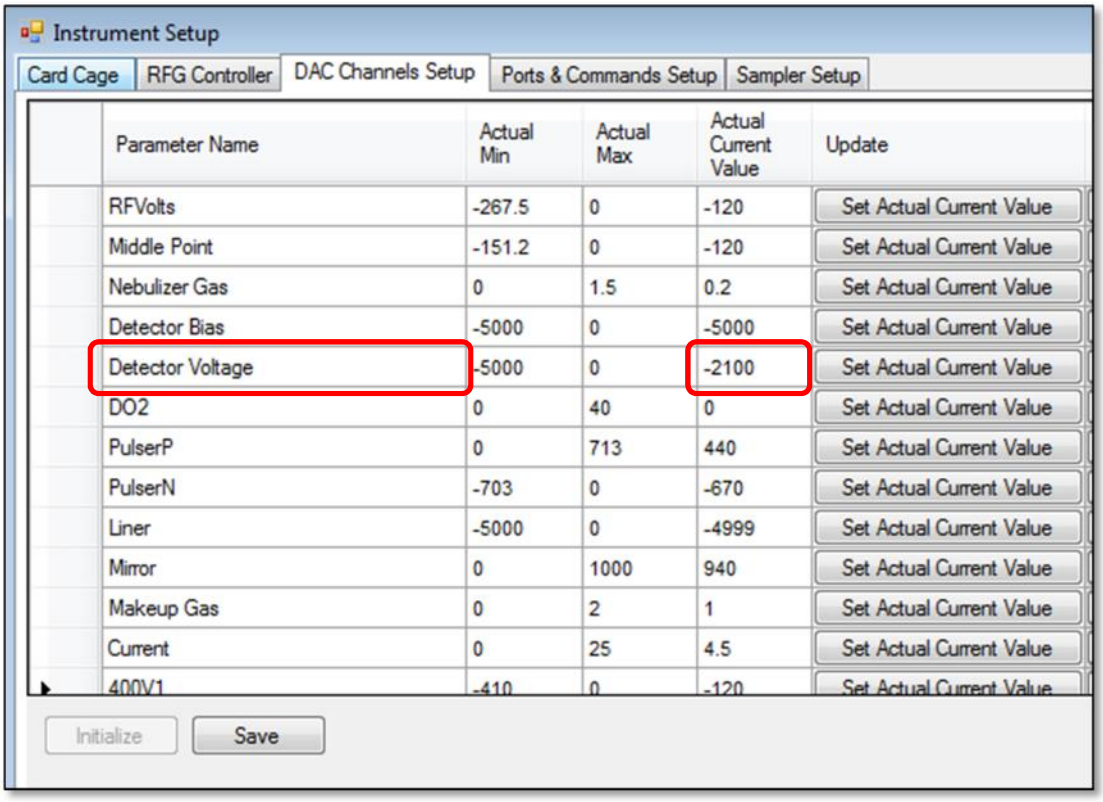
Dual Pulse Calibration and Detector Voltage Optimization

1. Select Tuning > Profile, right-click and select New Calibration.
2. Select **DV Optimization** in the General Parameters tab. **Dual Pulse Calibration is selected by Default.**



3. Inject 500uL of Tuning Solution, and click “Run” in the Control tab.
4. When the run is finished, note the Optimal DV from the Results Tab in Auto-Tuning window.
5. In Setup

>DAC Channels, enter this value into “Actual Current Value” and click “Set Actual Current Value” and “Save”.


Instrument Setup
Card Cage
RFG Controller
DAC Channels Setup
Ports & Commands Setup
Sampler Setup
Parameter Name
Actual Min
Actual Max
Actual Current Value
Update
RFVolts -267.5 0 -120 Set Actual Current Value
Middle Point -151.2 0 -120 Set Actual Current Value
Nebulizer Gas 0 1.5 0.2 Set Actual Current Value
Detector Bias -5000 0 -5000 Set Actual Current Value
Detector Voltage -5000 0 -2100 Set Actual Current Value
DO2 0 40 0 Set Actual Current Value
PulserP 0 713 440 Set Actual Current Value
PulserN -703 0 -670 Set Actual Current Value
Liner -5000 0 -4999 Set Actual Current Value
Mirror 0 1000 940 Set Actual Current Value
Makeup Gas 0 2 1 Set Actual Current Value
Current 0 25 4.5 Set Actual Current Value
400V1 -410 0 -120 Set Actual Current Value
Initialize
Save

Makeup Gas and Nebulizer Gas

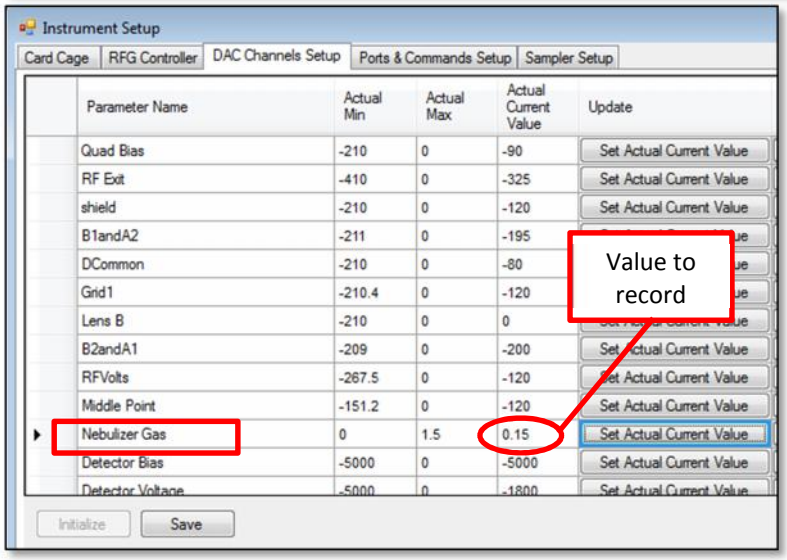
Note: Only perform this tuning step if either:

- ^{159}Tb Dual Counts are significantly lower than the previous well performing operating session (>400K with 204800 pushes per reading), OR
- ^{155}Gd/^{159}Tb ratio is at 3% or above.

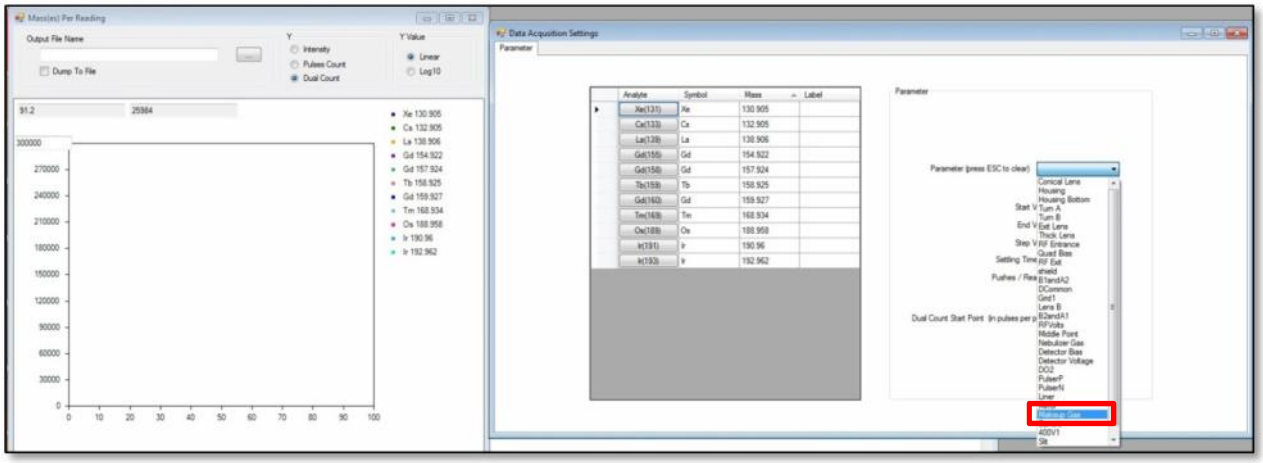
If necessary, tune Makeup and Nebulizer gasses according to the following protocol:

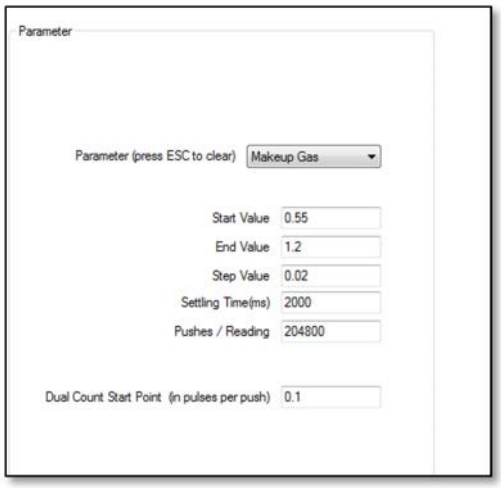
1. In **Instrument Setup** >**DAC Channel Setup**, go to Nebulizer Gas and take note of the current Nebulizer Gas value. Record value in the "Gas_Current_X-Y" worksheet in the CyTOF2 Manual Tuning Log.

Date	Nebulizer Gas Value	Optimal Make Up Gas Value	Tb159 Dual Count	Mass155 Dual Count
4/26/2013	0.15			

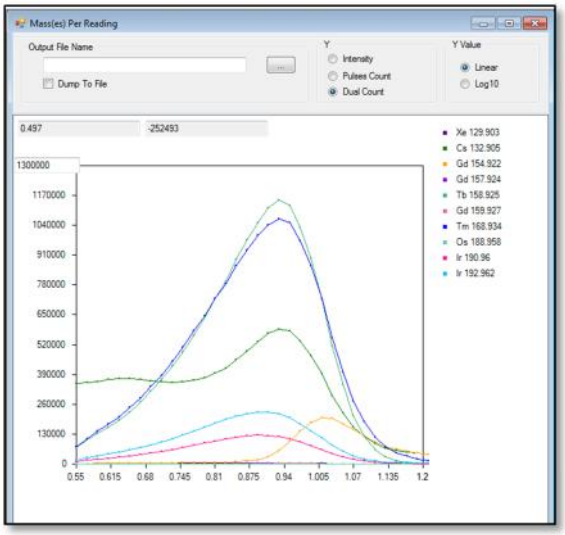

Instrument Setup
Card Cage
RFG Controller
DAC Channels Setup
Ports & Commands Setup
Sampler Setup
Parameter Name
Actual Min
Actual Max
Actual Current Value
Update
Quad Bias -210 0 -90 Set Actual Current Value
RF Exit -410 0 -325 Set Actual Current Value
shield -210 0 -120 Set Actual Current Value
B1andA2 -211 0 -195
DCommon -210 0 -80
Grid1 -210.4 0 -120
Lens B -210 0 0
B2andA1 -209 0 -200
RFVolts -267.5 0 -120
Middle Point -151.2 0 -120 Set Actual Current Value
Nebulizer Gas 0 1.5 0.15 Set Actual Current Value
Detector Bias -5000 0 -5000 Set Actual Current Value
Value to record
Initialize
Save

2. Open the **Mass Graph** window and **Data Acquisition Settings** window . Arrange the windows so that both windows are easily accessible.





3. Select Makeup Gas and enter the parameters as shown above.
4. In the Mass Graph window, Select "**Dual Count**" for the Y-axis and set the maximum count for the Y-axis to an appropriate value for the instrument.
5. Inject 500uL of tuning solution, and click "Run" .



6. Select the Makeup Gas value at which ^{159}Tb Dual Count is at maximum when ratio of Mass ^{155}Gd/^{159}Tb is <3%.
7. Record this value in the Gas Flow Optimization Log worksheet in a CyTOF QC Log File in the following format:

Date	Nebulizer Gas Value	Optimal Make Up Gas Value	Tb159 Dual Count	Mass155 Dual Count	Mass155/Tb159 %
4/26/2013	0.15	0.82	620998	6780	1.091790956

8. Repeat this process for Makeup Gas for different Nebulizer Gas values from +0.02 of initial set point up to +0.06. For example, with an initial set point of 0.15, ramp Makeup Gas at Nebulizer Gas settings of: 0.15, 0.17, 0.19, 0.21.

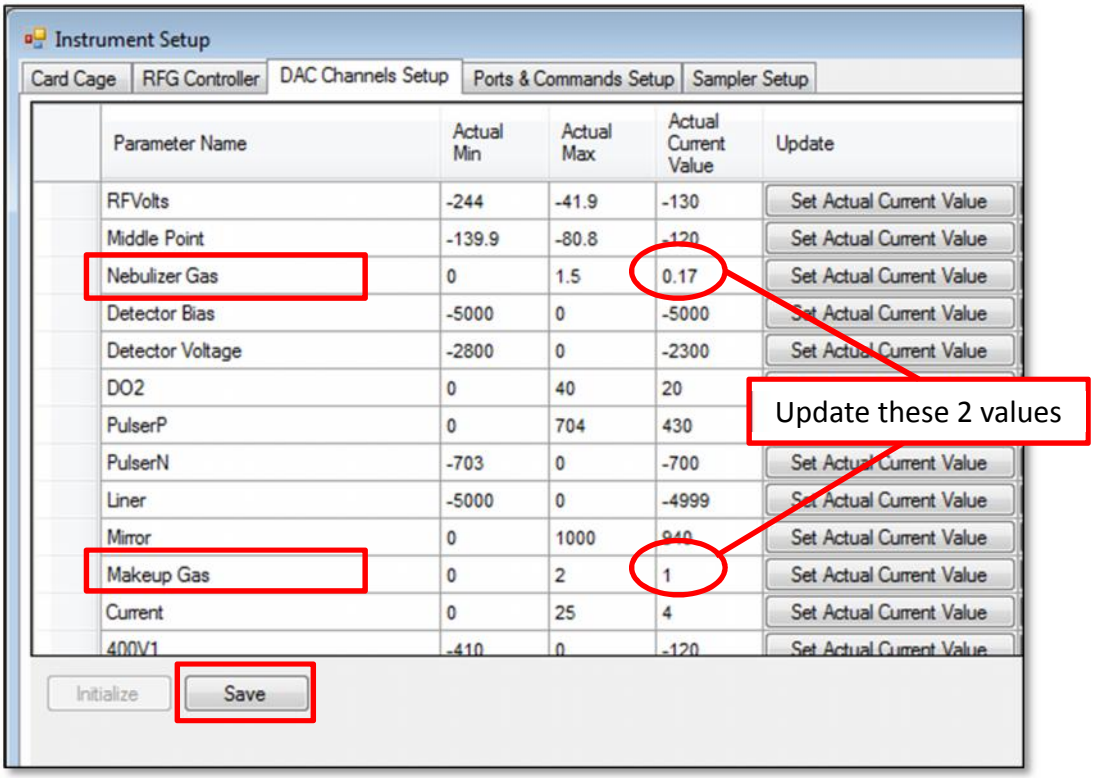
9. Fill in the data obtained at each Nebulizer Gas setting for Tb159 and Mass155 dual counts at each Optimal Makeup Gas value.

Note: If the ^{159}Tb Dual Count is comparable to the result from the day before, and the ratio of ^{155}Gd/^{159}Tb is lower than 3% after ramping Makeup Gas at existing Nebulizer Gas, you do not need to ramp again with different Nebulizer Gas settings.

Note: It is not necessary to lower Nebulizer Gas for the ramping, because over time the Nebulizer nozzle expands and it is unusual that lower Nebulizer Gas will give higher performance.

Nebulizer Gas Value (L/min)	Optimal Makeup Gas value (L/min)	Tb159 Dual count	Mass 155 Dual count	Mass155/Tb159
0.15	1.07	486,000	9300	1.6%
0.17	1.00	540,000	13500	2.5%
0.19	0.90	526,500	13700	2.6%
0.21	0.80	486,000	12500	2.5%

9. Using the Table just created, choose the combination of Nebulizer Gas and Makeup Gas where the ^{159}Tb signal is the highest as long as the ^{155}Gd/^{159}Tb ratio is below 3%.
   In this example (see figure above) the optimal combination is: Nebulizer Gas at 0.17 and Makeup Gas at 1.00.
10. Enter the gas values in **Instrument Setup** > **DAC Channel Setup** and click **Save.**
11. If ^{155}Gd to ^{159}Tb ratio does not go lower than 3%, try a new Nebulizer.

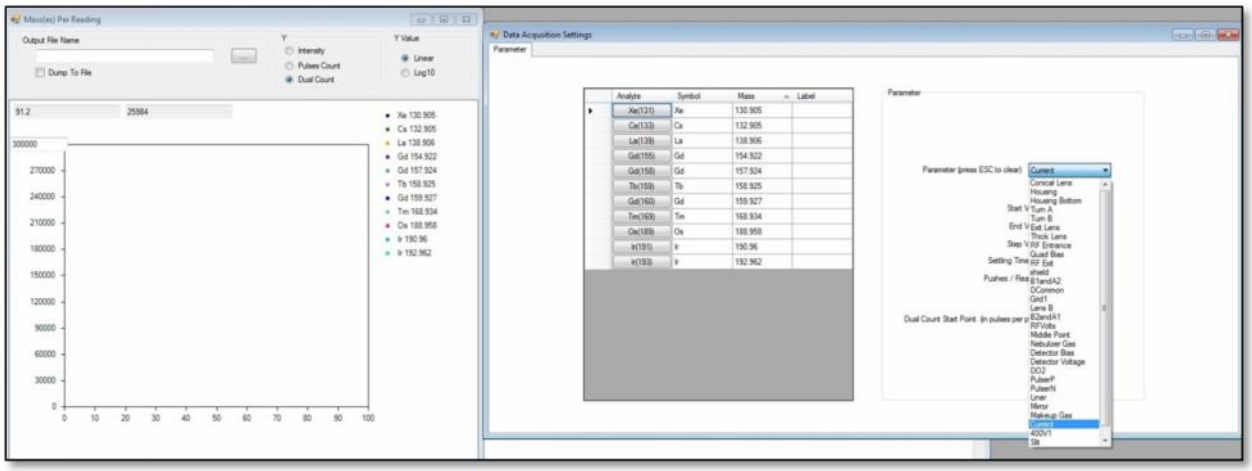


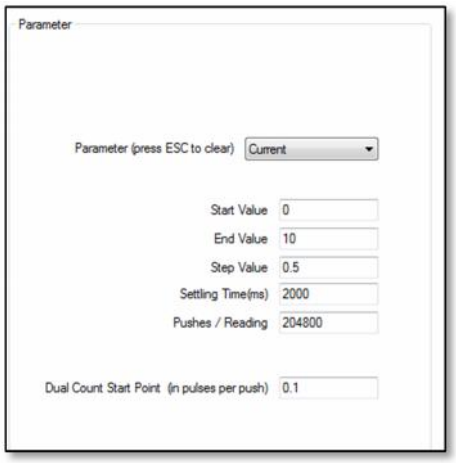
Current Optimization

Note: Only perform this tuning step if the ^{159}Tb Dual Counts are significantly lower than the previous well-performing operating session (>400K with 204800 pushes per reading).

If necessary, tune Current according to the following protocol:

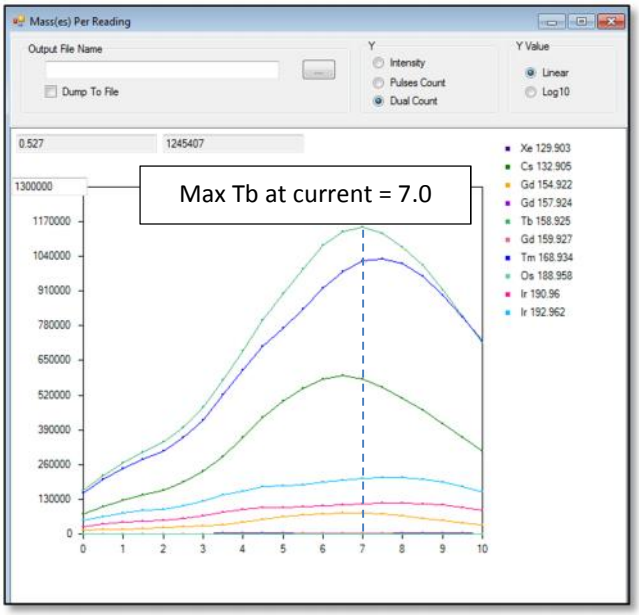
1. Open the Masses per Reading window and Data Acquisition Settings windows . Arrange the windows so that both windows are easily accessible.





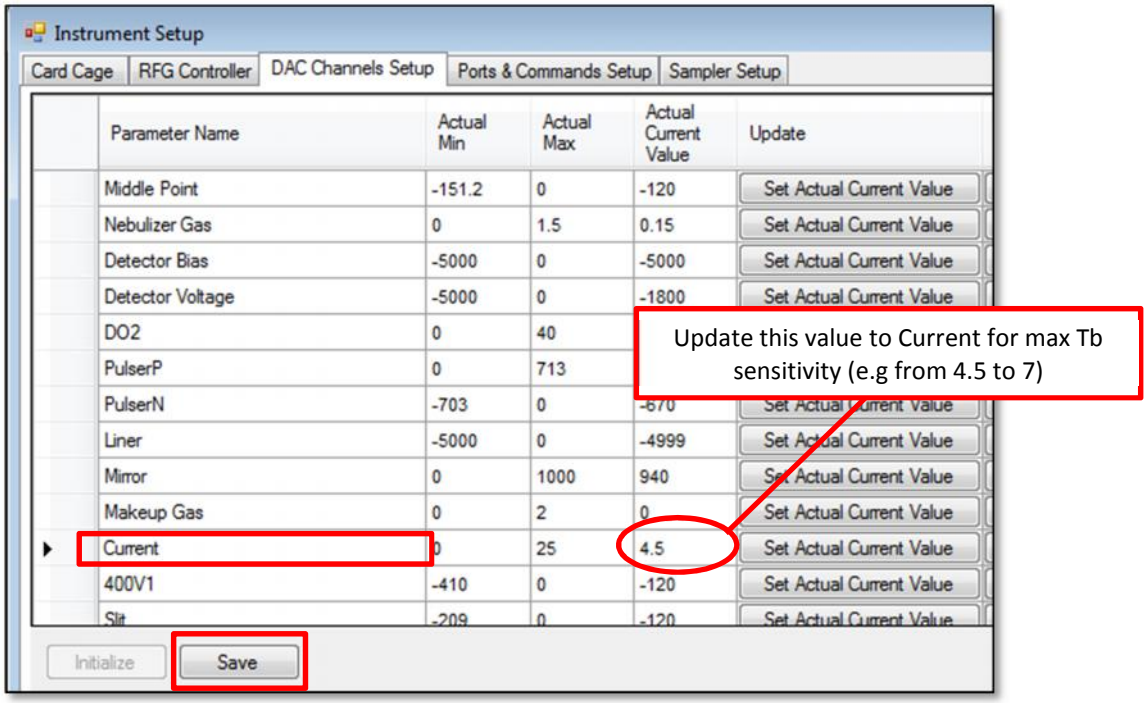
2. Select “Dual count” for the Y-axis and set the maximum count for the Y-axis to an appropriate value for the instrument.
3. Select Current and enter the parameters as shown above.

4. Inject 500uL of tuning solution, and click “Run” .



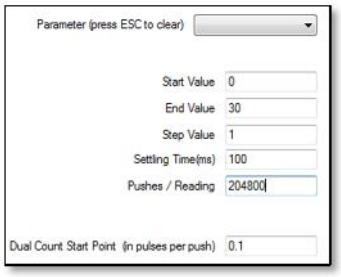
5. Choose the Current value for which the Tb Dual Count is at maximum.

6. Enter this Current value in the DAC Channel setup under Instrument Setup. Click on “Set Actual Current Value” and “Save”.


Instrument Setup
Card Cage
RFG Controller
DAC Channels Setup
Ports & Commands Setup
Sampler Setup
Parameter Name
Actual Min
Actual Max
Actual Current Value
Update
Middle Point -151.2 0 -120 Set Actual Current Value
Nebulizer Gas 0 1.5 0.15 Set Actual Current Value
Detector Bias -5000 0 -5000 Set Actual Current Value
Detector Voltage -5000 0 -1800 Set Actual Current Value
DO2 0 40
PulserP 0 713
PulserN -703 0
Liner -5000 0 -4999 Set Actual Current Value
Mirror 0 1000 940 Set Actual Current Value
Makeup Gas 0 2 0 Set Actual Current Value
Current 0 25 4.5 Set Actual Current Value
400V1 -410 0 -120 Set Actual Current Value
Slit -209 0 -120 Set Actual Current Value
Initialize
Save
Update this value to Current for max Tb sensitivity (e.g from 4.5 to 7)

When Manual Tuning is Complete

1. Save a 30 second reading file for Masses per Reading Graph using the Tuning Solution analyte template with the following parameter settings.


Parameter (press ESC to clear)
Start Value 0
End Value 30
Step Value 1
Settling Time(ms) 100
Pushes / Reading 204800
Dual Count Start Point (in pulses per push) 0.1

The ^{159}Tb dual counts should be >400,000.

2. Record the following values in the CyTOF2 QC Log:

   From the DAC Channels Tab:
   a. Makeup Gas
   b. Nebulizer Gas
   c. Current

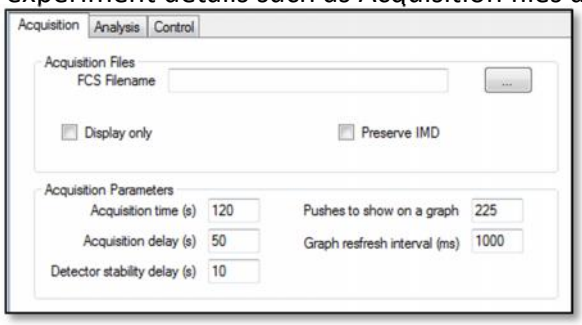
   From the Setup>X-Y Setup>Setup
   a. Current X-Y Values

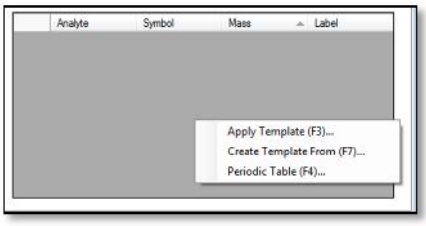
- **From the Active Auto-Tuning Profile > Results Tab**
   a. Optimal Detector Voltage (DV)

Bead Sensitivity Test

1. Open

, specify in the directory a location to save the file, and set up the experiment details such as Acquisition files and parameters as follows:





2. Right-click in the analyte table on the right to apply a template (F3), or to make a new template with the periodic table and save the template by selecting “Create Template From (F7)”.
3. Use the default settings in the Analysis Parameters Tab.
4. Inject 500uL of beads, and click Run in the Control Tab. Once the acquisition finishes, observe the data in a third party FCS file reader.
5. Gate singlet population and doublet population. Add event # of singlets to event # x 2 of doublets. This total should be at least 12000 events. If not, rerun beads.
6. Check that the mean of singlet population for ^{151}Eu or ^{153}Eu is at least 1000. If not, rerun beads.

Cleaning After Tuning

Tuning Solution

1. To clean the loops after the Tuning Solution is run, inject 1mL of Washing Solution and click “Preview” .
2. Allow Washing Solution to run for 2-5 minutes.
3. Repeat for the other loop.
4. Repeat for both loops with DIW.
5. Allow DIW to run for 2-5 minutes before proceeding.

Beads

1. To clean the loops after beads are run, inject 1mL of DIW and click “Preview” in the control tab. This will display 10 snapshots of any ion signal traces that are detected.

2. Allow DIW to run through loop for 2 to 5 minutes and click “Preview” to check for residual beads.
3. To clean the second loop, inject 1mL of DIW and click “Preview” again..
4. Allow DIW to run through loop for 2 to 5 minutes and click “Preview” to check for residual beads.
5. If the beads are persistent in the loops, inject 500uL of Washing Solution and click ”Preview”.
6. Allow Washing Solution to run for 2 to 5 minutes and then repeat for the second loop.
7. Run DIW for 2-5 minutes through each loop after running Washing Solution.
8. Click ”Preview” to check status before proceeding.

Sample Acquisition

Sample Preparation

Please refer to DVS protocols for sample preparation.

Before Acquisition

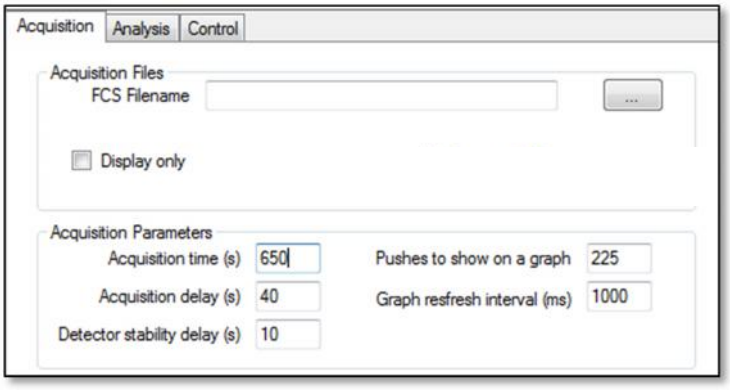
It is strongly recommended that users add diluted **CyTOF Calibration beads** to samples as an internal standard:

1. Vigorously shake the bottle with Calibration Beads. Then dilute the Calibration Beads 1/10 in deionized water.
2. Add the diluted Calibration Beads directly into the vial with the pelleted sample, and mix well. This will be the sample for acquisition.
3. Normalization of cell data after acquisition can be done by dividing the signal value from the marker of interest by Eu signal value in the same sample. This resulting normalized signal is independent of any intrinsic variability of the instrument. The Normalization tool (available from your FAS) can also be used.

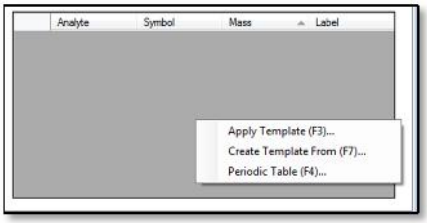
Set up Acquisition Parameters and Sample Introduction

1. To run samples: open the **Acquisition** window from

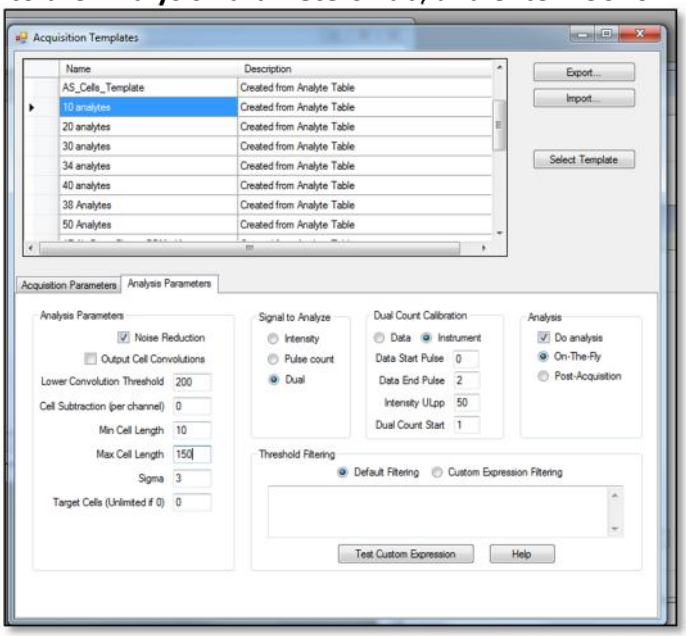
in the menu bar.
2. Specify a pathway and filename to save an FCS file.
3. Setup Acquisition Parameters.
    a. Acquisition time is the duration of the sample acquisition in seconds. When the default syringe speed of 0.045 mL/min is used, it will take approximately 650 seconds (more accurately 667s) to collect the 500 µL Sample Loop volume.
    b. The acquisition delay is typically 40 sec.
    c. Detector stability delay should be set to 10 sec.



4. Right-click in the Analyte table (shown below) to apply a template (F3), or to make a new template with the periodic table.
5. Save the template by selecting “Create Template From (F7)”.



6. The **Acquisition Templates** window will then open with the selected analytes saved. Go to the **Analysis Parameters** Tab, and enter 150 for **Maximum Cell Length.**



7. Enter the number of events you wish to collect in “**Target Cells (Unlimited if 0)**”. If you do not wish to set the number of cells to be acquired and instead run for a specified time, you must enter “0” in the **Found Cells Limit** box. Use the default settings for other parameters in the Analysis Parameters Tab.

   Note: Some settings in this tab can also be found in the **Analysis** tab in the **Acquisition** window outside of the **Acquisition Templates** window. It is recommended to make changes in the **Acquisition Templates** window to ensure that the parameters are consistent across multiple samples in the same experiment.

8. Settings are saved automatically once you navigate away. Click on **Select Template** to exit this view and return to starting sample acquisitions.
9. Inject 500 µl of your sample into the injection port and click “Run” in the Control tab.
10. Once the acquisition finishes, observe the data in Plotviewer, if desired.

Daily Cleaning

Cleaning During Operation

Cleaning between samples

1. Push 1-3 mL of MilliQ or equivalent water (DIW) through the Sample Loop.
2. Click ”Preview”. Leave for 2-5 minutes while DIW from the carrier reservoir is running through the loop.
3. Repeat for the other loop.
4. Check background signal using “Preview”.
    a. If background signal has returned to baseline, proceed to the next sample.
    b. If background signal is high, inject 1 mL of Washing Solution into the Sample Loop and click “Preview”.
    c. Repeat for the second loop.
    d. Allow Washing Solution to run for at least one minute and monitor with “Preview”.
    e. Flush DIW through both loops before proceeding to the next sample.

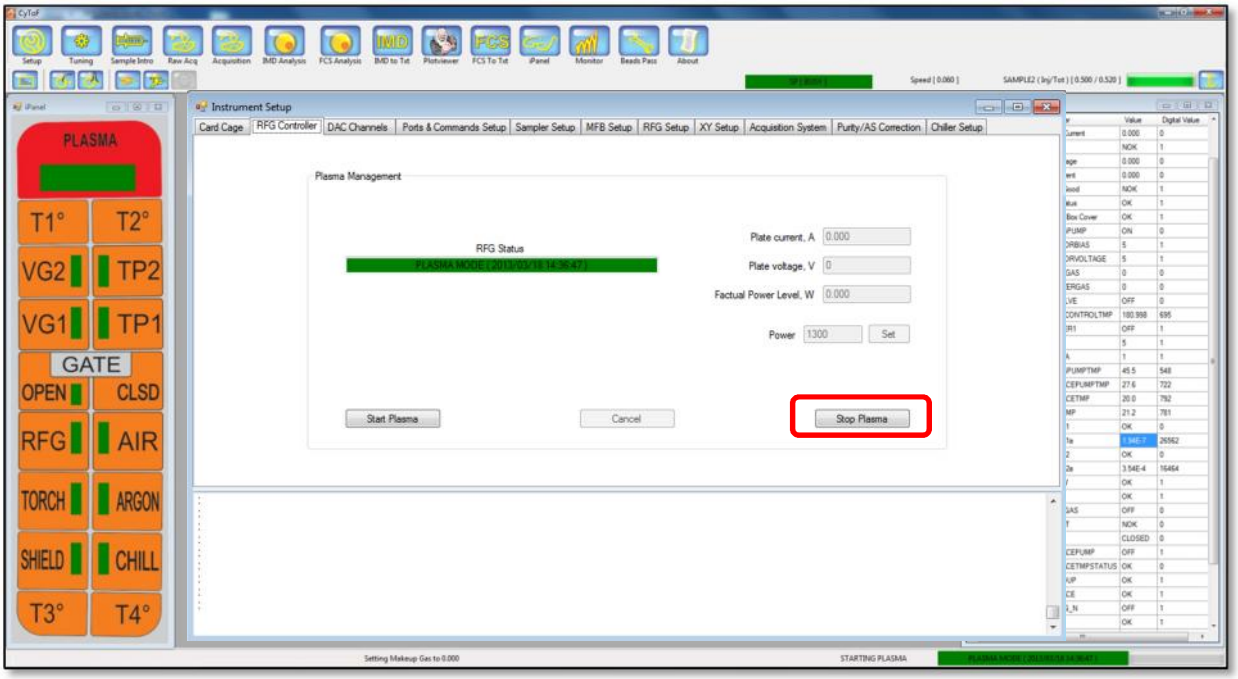
Cleaning between different users or experiments, and at end of the day.

1. Push 1 mL of Washing Solution through the Sample Loop.
2. Click “Preview” and let run for 2-5 minutes.
3. Repeat Steps 1 & 2 for the second loop.
4. Repeat Steps 1 & 2 with 1 mL of DIW for each loop.
5. Click “Preview” again.
6. Repeat steps 1-4 if background signal has not returned to baseline.

Shutdown: Turning Off Plasma

1. In **Setup**

> **RFG Controller** > click **Stop Plasma**



2. Wait until the "Plasma Stop Sequence has been completed successfully" message appears (see below). The Syringe Pump, Chiller and Heater will automatically be turned off when the Plasma Stop Sequence is completed.

3. Remove the Sample Capillary from Nebulizer and then the Nebulizer from the Nebulizer Port.
4. Disconnect the Nebulizer from the gas line.
5. Using the syringe plus tubing tool, slowly pull 10% Contrad or Decon 90 into the Nebulizer and soak for 15 min.
6. Rinse the Nebulizer 2 to 3 times with DIW using the syringe and tubing tool.

Note: When pushing liquid out of the Nebulizer, only apply enough pressure so that residual liquid drips from the Nebulizer tip. Do not use enough force to form a steady stream.

7. Leave the Nebulizer submerged in a DIW bath prior to next use.

Consumables

Spare Parts

The CyTOF 2 instrument comes with 1 spare of each part listed below. The suggested total number of each spare part to have available is indicated in red:

Spare Part	Cat#	Spares Included	Additional Spares Recommended*
Nebulizer	101794	1	2
Torch Body	101792	1	1
Ball Joint Injector	101542	1	1
Spray Chamber	105545	1	1
Sample Capillary Assembly	101519	1	1
Load Coil	105398	1	0
Sample Pump Tubing Kit	101935	1	1
Skimmer-Reducer Assembly	101802	0	1
Sampler Cone	105197	0	1
Nebulizer Arm O-rings	101817	0	1 pack of 5
Nebulizer Arm Ferrule	101933	0	1 pack of 5

* To order additional parts, visit the DVS web catalog (http://www.dvssciences.com/product-catalog-metal.php) and click on the 'CyTOF Reagents and Spare Parts' tab.

Reagents and Labware

- Henke Sass Wolf 1ml and 3ml sterile NormJect® luer syringes, available from various vendors including Chem Glass Life Sciences, Henke Sass Wolfe Gmbh and Agro Weber (rubber-free).
- Falcon™ 5ml polypropylene tube with cell strainer cap (35 µm), catalog # 352235.
- Calcium- and Magnesium-free PBS, available from various vendors.
- High-grade 18Mohm De-ionized water (DIW), e.g. Milli-Q from Millipore.
- Glassware and plastics: polypropylene or Pyrex is recommended, rather than glass, to minimize Lead contamination. Avoid contact with detergents which may be a source of Barium.
- General reagents should be of analytical grade.

Chapter 6
Maintenance

Instrument cleaning and maintenance ensures optimal operational performance of your CyTOF® 2 instrument. Table 6.1 summarizes routine cleaning and other required maintenance. Subsequent sections will detail how these procedures are performed.

Table 6.1 Summary of Routine Cleaning and Maintenance

Part	Frequency	Responsible Party	Necessary Reagents
Nebulizer	Daily	Operator	10% Contrad 100 or 10% Decon 90 Deionized Water (DIW)
Spray Chamber **Injector** **Torch Body**	Weekly	Operator	10% Contrad 100 or 10% Decon 90 DIW
Cones (Sampler and Skimmer Reducer)	Daily-Weekly (depending on Instrument usage)	Operator	10% Citranox 2% Nitric Acid (optional) DIW
Load Coil	Weekly	Operator	Ultrapure Methanol
Interface Pump Oil	Annually or as needed	Engineer once per year /Operator	HE-100 vacuum oil
Backing Pump Oil	Every 6 months	Engineer once per year /Operator	HE-100 vacuum oil
Air Filters	Annually	Field Service Engineer	N/A

Required Materials

1. Sonicator
2. Cleaning Solutions
    a. Contrad® 100 or Decon 90 (Decon Labs, Cat #1504 1 gallon): dilute to 10% in MilliQ grade water (DIW).
    b. Citranox® Liquid Acid Cleaner and Detergent (Alconox, Cat# 1801 4 x 1 gallon or Sigma-Aldrich, Cat# Z273236-1 ea, 3.7L): dilute to 10% in DIW.
    c. Nitric Acid, (Seastar Chemicals Inc. Cat# S020101): dilute to 2% in DIW. US Suppliers: VWR: BDH Aristar Ultra Nitric Acid; Thermo Fisher: Optima Nitric Acid

d. Ultrapure Methanol.

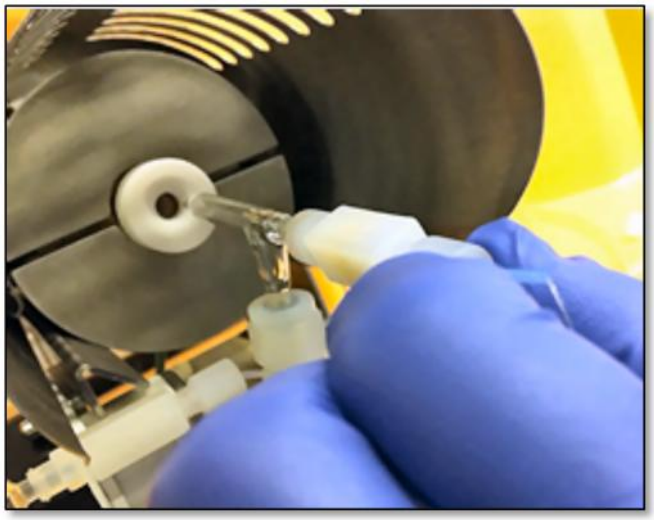
3. Tools (dedicated to cleaning CyTOF parts only)
    a. Glassware brushes of varying sizes.
    b. Scotch-Brite Ultra-fine Hand Pad (3M 7448).

Cleaning Between Samples and Prior to Plasma Shutdown

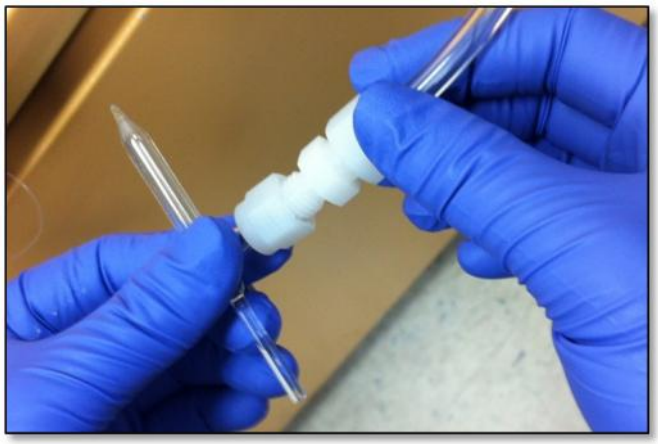
Nebulizer

Timely removal of the Nebulizer from the elevated temperature environment of the Heater Module as well as soaking in DIW when not in use will help to avoid clogging of the tip.

1. Remove the sample capillary tubing and set aside.
2. Remove the Nebulizer from the Nebulizer Adapter.



3. Loosen the connection of the Nebulizer gas and remove the Nebulizer.



4. Retighten the union being careful to retain the O-ring and ferrule.
5. Connect the side arm of the Nebulizer to the syringe and tubing tool.
6. With the Nebulizer tip submerged in detergent, pull slowly on the syringe plunger to fill the nebulizer with detergent.

7. Repeat steps 5 & 6 with the syringe and tubing tool connected to the sample inlet.

8. Soak the Nebulizer in detergent for 15 min.
9. Pull back on the syringe plunger to draw air into the syringe.
10. Attach the syringe and tubing tool to the nebulizer side arm and slowly push on the plunger to expel detergent from the Nebulizer into a waste beaker.

Note: Detergent should drip out. If the detergent comes out in a stream, the syringe is being depressed too quickly.

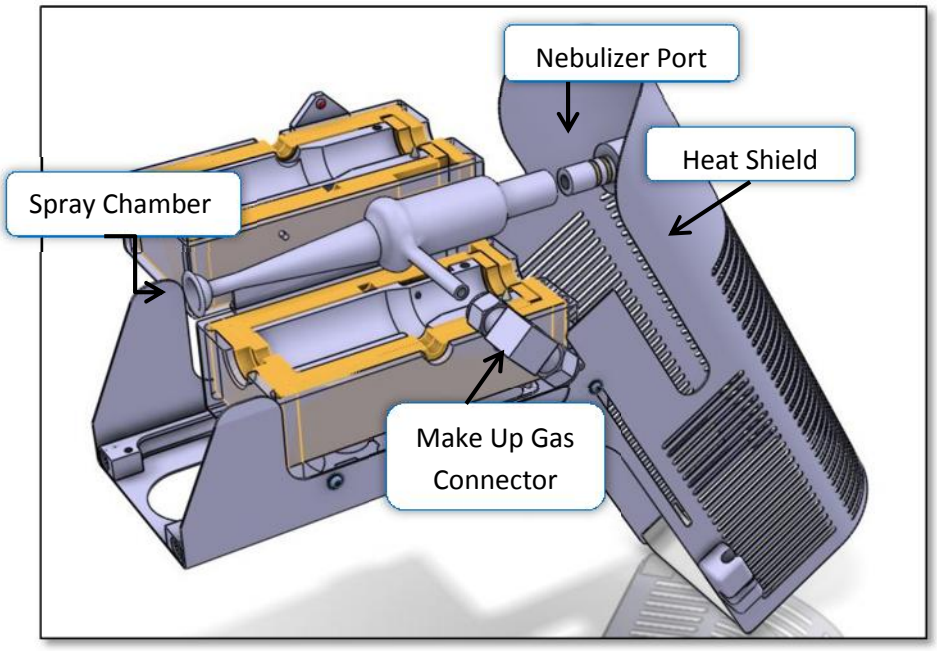
11. Use the syringe and tubing tool on both the side arm and sample inlet to rinse the Nebulizer with DIW. Repeat the rinse 2-3 times.
12. Leave the Nebulizer soaking in DIW until next use.

Maintenance of the Spray Chamber and the Torch Assembly

Allow heater to cool for at least 30 min after plasma shutdown before attempting disassembly.

Removal of the Spray Chamber

1. Slide the Heat Shield off the Heater.
2. Remove the ball joint clamp which secures the Spray Chamber to the Injector.
3. Open the Heater lid.
4. Loosen the Make Up gas line from the Spray Chamber sidearm.



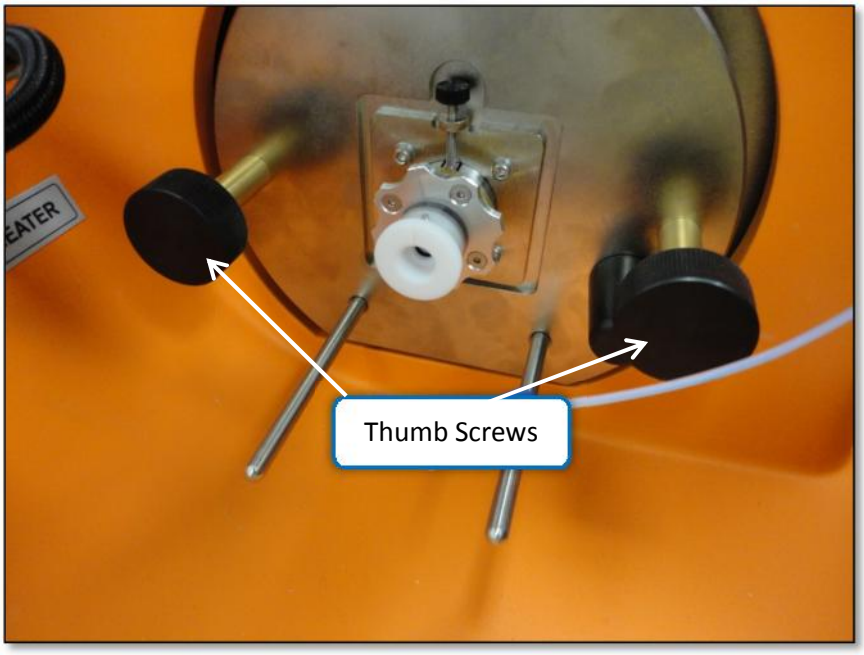
5. Remove the Spray Chamber from the Heater and remove the Nebulizer Port.
6. Slide the entire Heater module off the guide pins and then rest on the pins below the drip tray.

7. Remove the Ball Joint Injector by gently pulling and turning until it comes loose from the Torch Assembly.

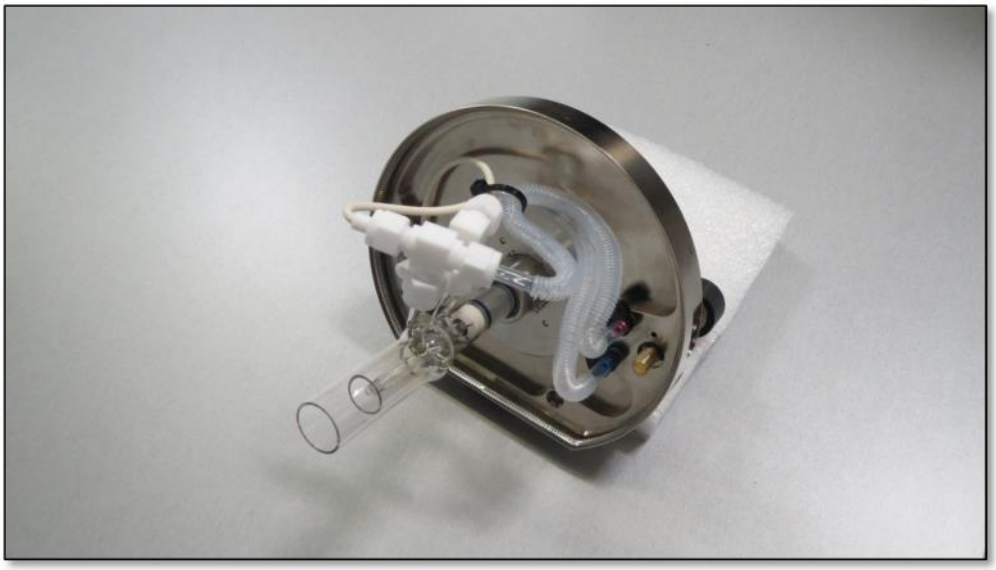
Disassembly of the Torch Body

WARNING: Before proceeding to step 1 below, switch OFF the RF generator power using the breaker, located at the right rear of the CyTOF2 instrument. Wait at least 5 minutes for residual electrical charge to dissipate. Additional time is required to allow the ICP torch, cones and the load coil to reach room temperature.

1. Loosen the two thumb screws at the front of the Torch Assembly making sure to loosen in unison.



2. Slide the Torch Assembly off the Torch Box pins to access the Torch Body.

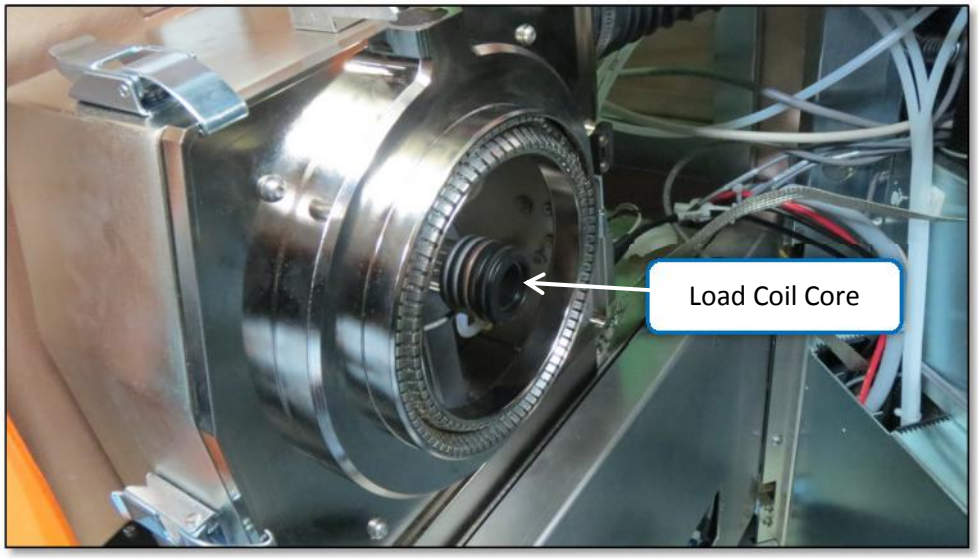


3. Carefully grasp the Torch with one hand and firmly hold the Torch Assembly with the other hand. Twist and pull the torch until it is free of the Torch holder.

4. Clean the Spray Chamber, Injector, and Torch Body as detailed in Table 7.1.
5. Let glassware dry completely before reassembly.

Cleaning the Load Coil

1. Install the Load Coil Core.



2. Using a Scotch-Brite Ultra-fine Hand Pad moistened with ultrapure methanol, gently rub the surfaces of the load coil to remove any deposits.
3. Remove the Load Coil Core.
4. Gently clean in between the coils with the hand pad and methanol being careful not to bend the coils.

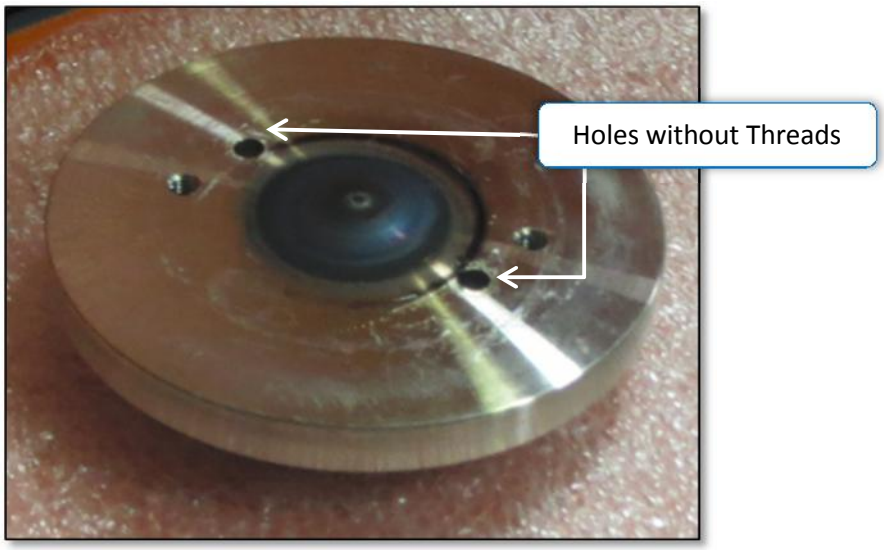
Removal of the Cones

Note: The Torch Assembly should be removed before removing the cones.

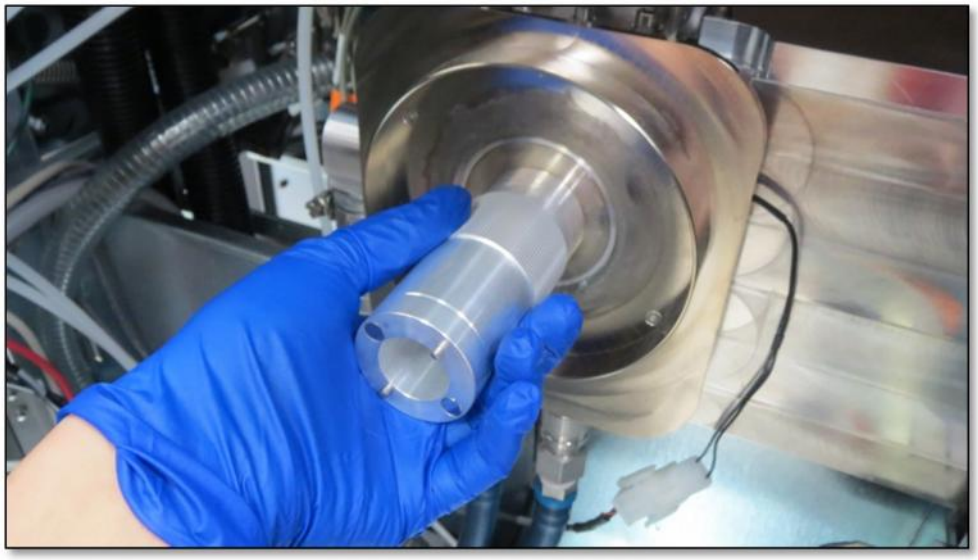
The cone removal tool contains magnets and pins that allow controlled removal of the sampler and skimmer reducer.

Sampler Cone

1. The Sampler cone face has four holes. The two without threads which lie closer to the Sampler orifice are used for removal.



2. Line up the pins on the cone removal tool with the non-threaded holes being careful not to touch the orifice.



3. Rotate the cone removal tool while pulling forward to release the Sampler from the vacuum.

4. Remove the O-ring from the Sampler.

5. Remove the Sampler cone from the Cone Removal Tool being careful not to come in contact with the Sampler orifice.

Skimmer Reducer

1. Using the other side of the Cone Removal Tool (two magnets), line up the pins with the two holes of the Skimmer Reducer.
2. Turn the Cone Removal Tool counter clockwise until the Skimmer Reducer comes free.
3. Remove the Skimmer Reducer from the cone removal tool, being careful not to touch the orifice.

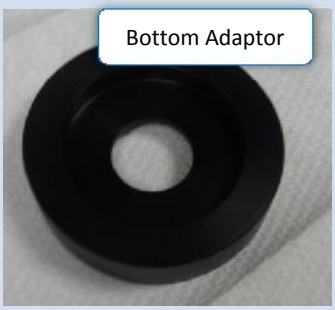
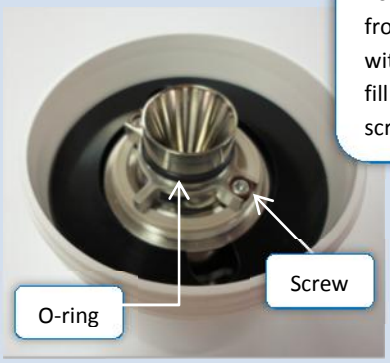
Cleaning of the Cones

During routine maintenance and cleaning, inspect the shape of the orifice and for deposits around the orifices of the Sampler and Skimmer/Reducer cones.

Important Note: During all steps of the cleaning process, care should be taken that nothing comes in contact with the orifices of the cones.

The cones should be stacked inside the Cone Cleaning Container using the included adaptors as shown in the table below.

Table 6.2 Adaptors and Cones

Step	Image	Step	Image
Step 1: Place Bottom Adapter inside the cone cleaning container (container not shown)		Step 2: Remove Sampler O-ring, then place Sampler Cone on top of Bottom Adapter	
Step 3: Place Top Adaptor on top of the Sampler Cone		Step 4: Place the Skimmer Cone on top of the Top Adapter	
Cones stacked inside Cleaning Container	 To prevent the O-ring from coming in contact with the Citranox, do not fill above the level of the screws on the Skimmer Screw O-ring		

1. Insert the cones and adaptors into the Cone Cleaning Container as described above, adding 10% Citranox at each step. Sonicate for 15 min.
2. Rinse with DIW.
3. Optional. For more aggressive cleaning of the cones, repeat step 1 using 2% Nitric Acid solution and then rinse with DIW.
4. Repeat step 1 twice using DIW.

5. Air-dry completely before reinstalling.

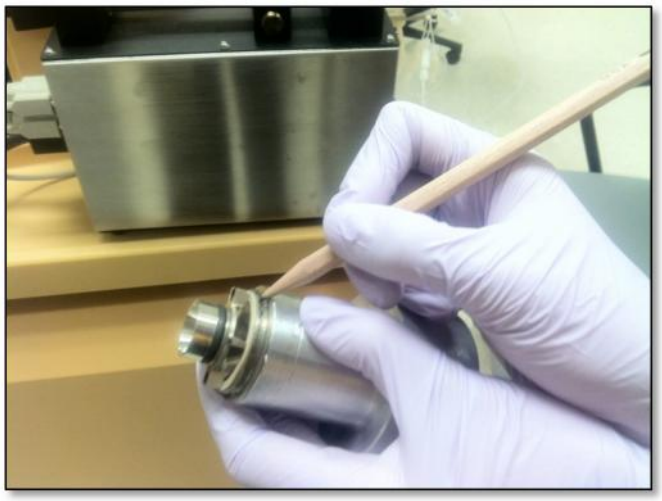
Note: The concentration of cleaning solutions, sonication times and frequency of cleaning are a guide only and can be modified for the best workflow that suits the user's needs.

Reinsertion of the Cones

Note: Always install the cones before installing the Torch Assembly.

Skimmer Reducer

1. Place the Skimmer Reducer on the side of the Cone Removal Tool with two magnets. With a No. 2 pencil, coat the threads of the Skimmer Reducer with graphite. This allows for easier threading of the Skimmer Reducer into the interface.



2. Making sure that the Skimmer Reducer is seated flush in the interface, begin to turn clockwise. After several turns, turn back a quarter turn to make sure mis-threading has not occurred. If there is no resistance while turning back, then continue turning clockwise.
3. Repeat step 2 until the Skimmer Reducer is firmly seated. Do not over tighten.
4. Detach the Cone Removal Tool from installed Skimmer Reducer.

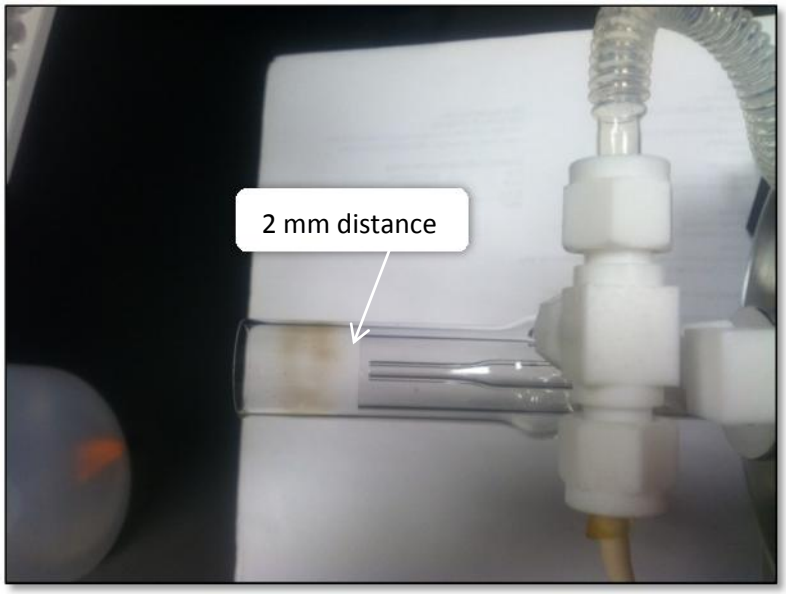
Sampler

1. Attach the Skimmer Reducer to the side of the Cone Removal Tool with four magnets.
2. Seat the Sampler flush in the interface. Make half a turn clockwise while applying gentle forward pressure.
3. Detach the Cone Removal Tool from the installed Sampler.
4. Press gently along outer edges of Sampler to make sure it is seated firmly.

Reassembly of the Torch

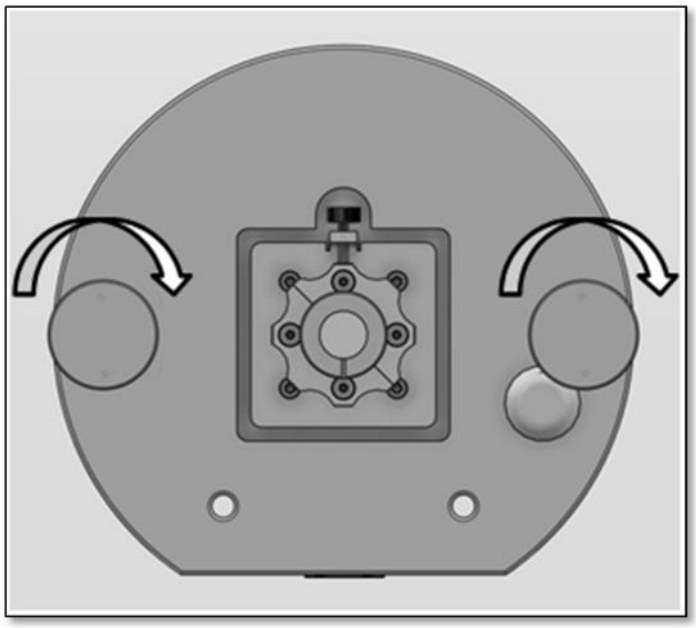
Note: Always install the cones before installing the Torch Assembly.

1. Install the Torch Body over the two O-rings of the Torch Holder by pushing and turning.
2. Turn the Torch Body so that the gas ports are oriented on top.
3. Connect the Auxiliary Gas line to the port closest to the Torch Holder. This port is slightly angled.
4. Connect the Plasma Gas line (with ignition pin) to the second port. This port is straight.
5. Ensure that both connections are tight.
6. Install the Ball Joint Injector by pushing and turning until it is fully inserted.
7. If the Torch and Injector are correctly installed, the Injector should be 1.5-2 mm from the end of the inner portion of the Torch.

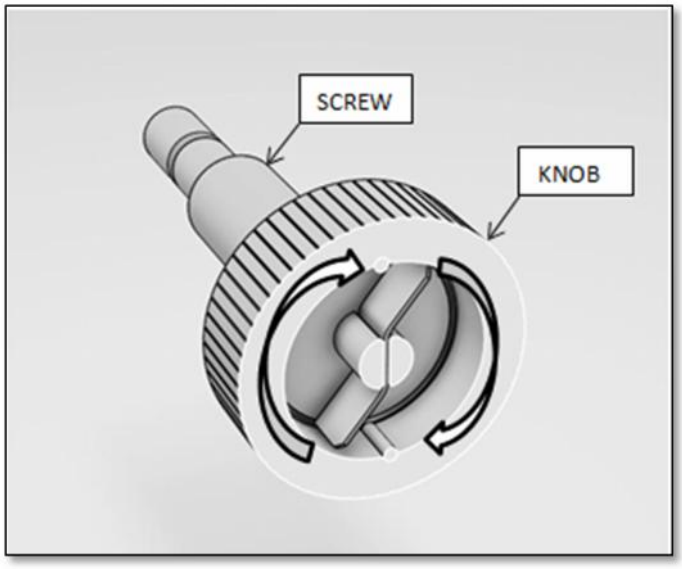


Installation of the Torch Assembly

1. With the CyTOF door closed, slide the Torch Assembly onto the Heater Box pins and push flush, making sure to line up the High Voltage Connector with its port.
2. When installing the Torch Assembly, ensure that both screws are rotated in unison.



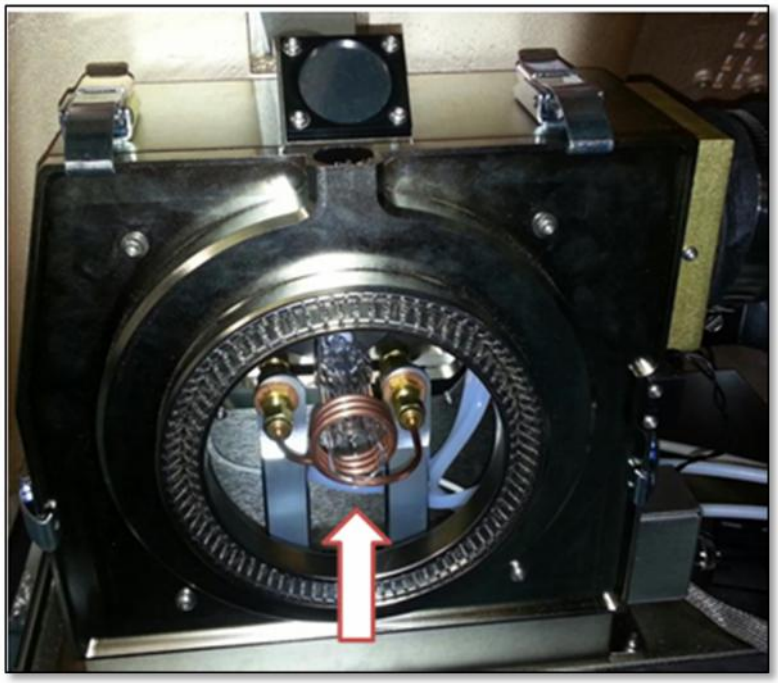
3. Note that the screws have an internal ratcheting system on the black knobs. Over a small range, these knobs are free to rotate without the brass screw being turned. Therefore, when installing/removing the Torch Assembly, always ensure that the knobs are moving in the same direction as the screw. Carefully preventing the knobs from accidentally rotating in the opposite direction can help ensure synchronized motion of the two screws during installation/removal.



4. When installing the torch holder, tighten the screws until an audible “click” is heard. This is to ensure that the torch holder installed properly in its end position.

Troubleshooting Installation of the Torch Assembly

1. In the event that the torch holder appears to be stuck on the instrument (during installation/removal), inspect the relative position of the screw assembly with respect to one another.
2. Open the front door of the instrument to be able to visualize the screw assembly from the inside.



3. Compare the amount of thread engagement between the two screw assemblies (the brass pieces shown below), then loosen or tighten the corresponding screws to equalize the thread engagement.

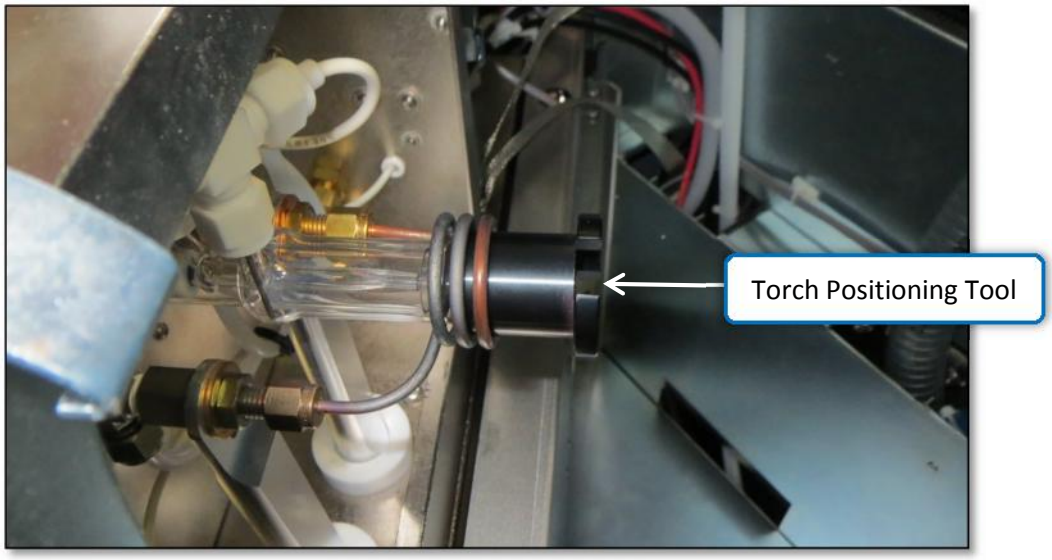
4. Turn the screws in sync to install/remove torch holder as required.

Checking the Torch Alignment

This process is necessary for two reasons. Firstly, it must be determined that the Torch is centered in the Load Coil. Secondly, the position of the Torch relative to the Interface (the Z-Alignment) must be checked. The distance from the Torch to the Interface needs to be correct so that ion clouds can travel optimally into the Cones.

Check that the Torch is centered in the Load Coil

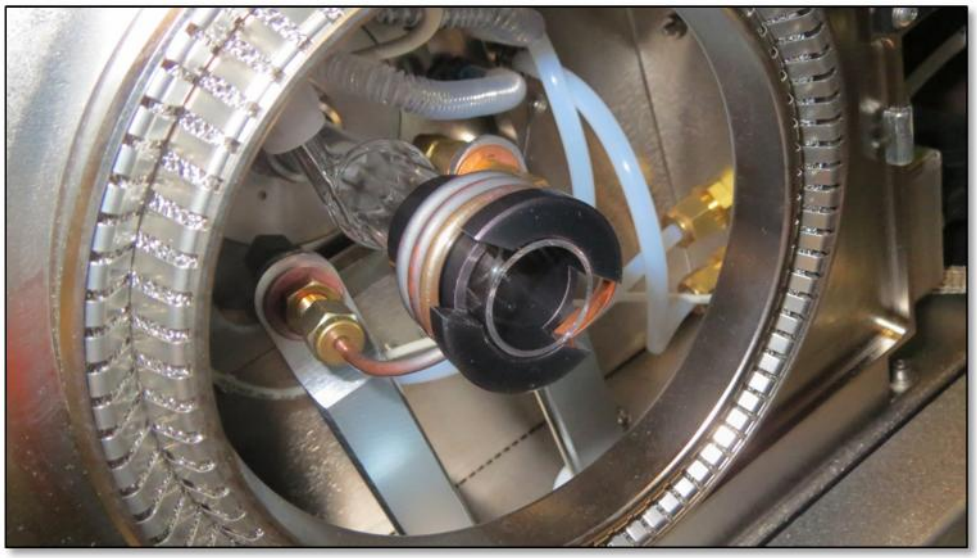
1. Open the CyTOF door.
2. Install the Torch Positioning Tool (may be either black or silver in color) in the end of the Torch.



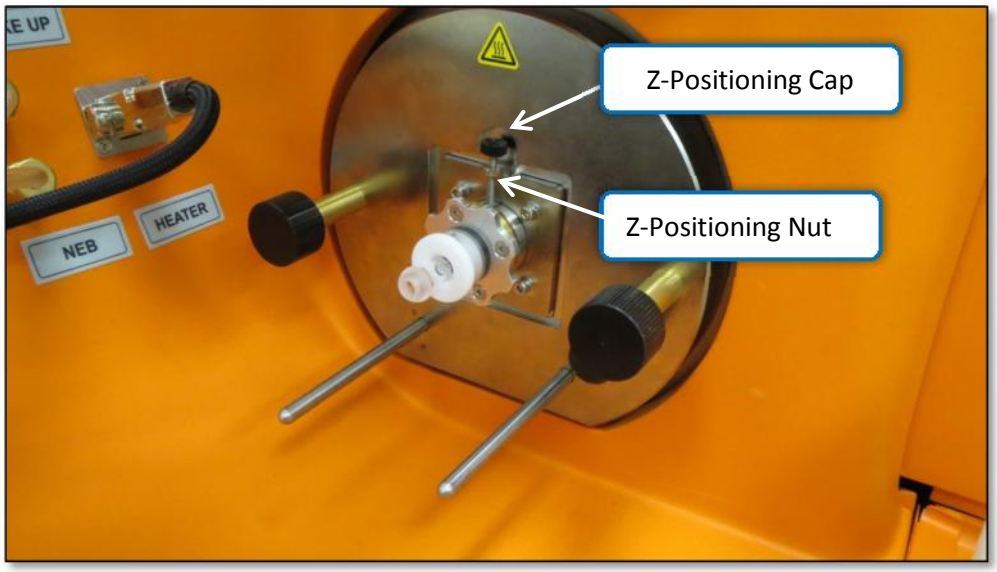
3. Turn the Torch Positioning Tool. It should spin freely.
4. If the tool doesn't spin freely, the Torch Box is not installed properly. Remove the Torch Assembly and reinstall following steps 6 & 7 in section "Reassembly of the Torch" above.

Check the Z-Alignment

1. Gently push the Torch Positioning Tool in as far as it will go.
   The outer edge of the Torch Positioning Tool should be flush with the edge of the torch.



Troubleshooting the Z-Alignment



1. Recheck the Z-Alignment as described above.

Instrument Air Filters

The CyTOF® 2 is equipped with a large and small air filter on the underside of the instrument. These air filters remove particles from the argon gas supply. The filters can be removed by opening the bottom of the instrument and pulling them out. These filters are inspected and changed by the DVS Sciences Service Engineers on an annual basis.

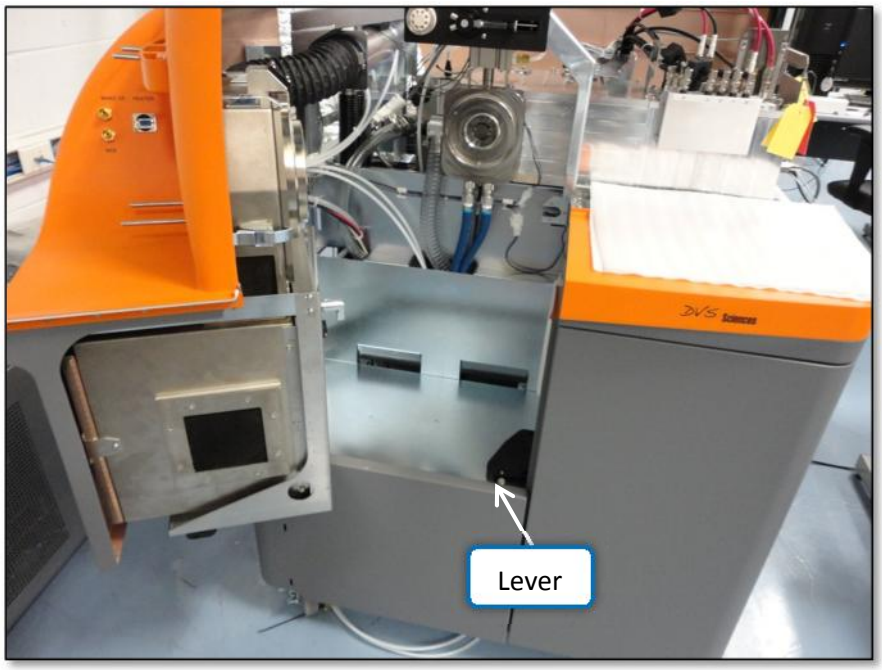
Rotary Pumps

The CyTOF® 2 has two rotary pumps, an interface pump and a backing pump. Maintenance of the vacuum pumps includes inspecting the pumps and changing the pump oil. Inspect the pump oil daily and compare the appearance of the oil with a small sample of new oil. Change the vacuum pump oil if it has an unusual color, is dark, contains particles, or appears dirty or turbid. Typically if the oil is the color of honey then it does not need changing. If it is a darker color then it should be changed immediately.

The following section details the procedure for changing the oil for both pumps.

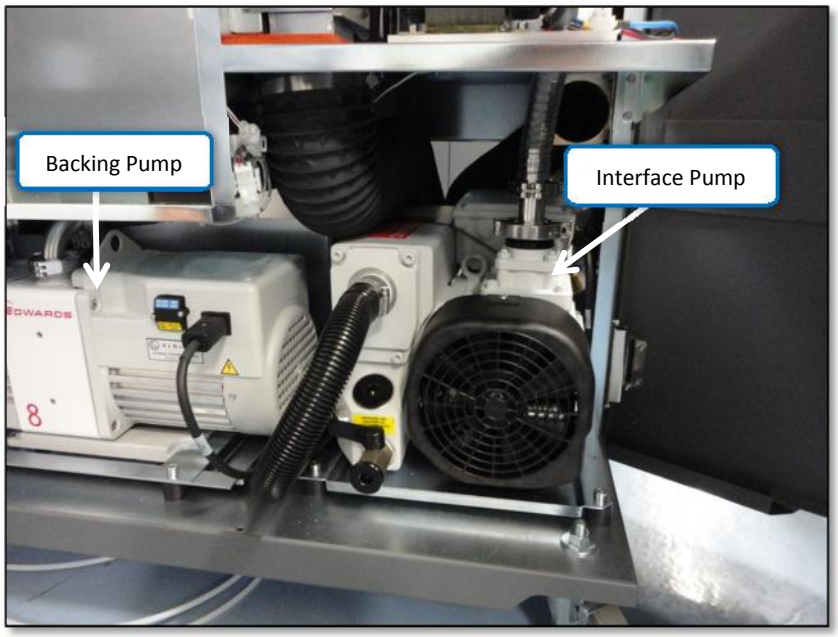
1. Turn off the vacuum pumps with the switch on the right side of the instrument.

2. After the vacuum pumps have completely shutoff, open the front access door.

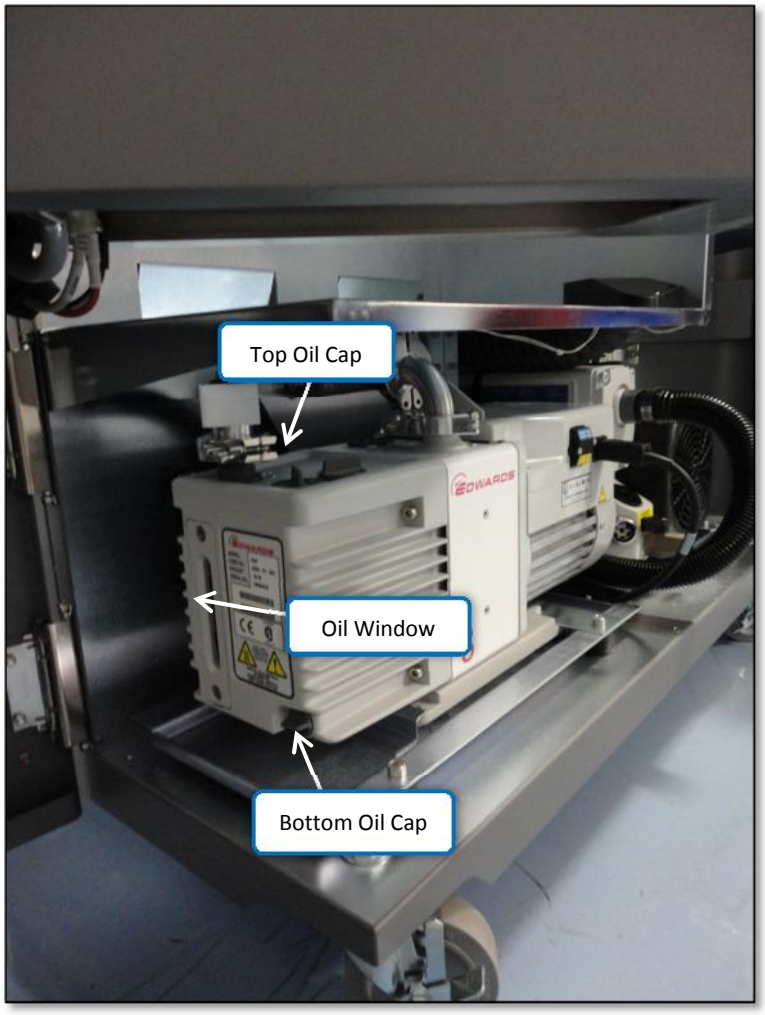


3. Pull the lever to the left to open the bottom compartment.

4. Open the door on the right front of the instrument to access both pumps.

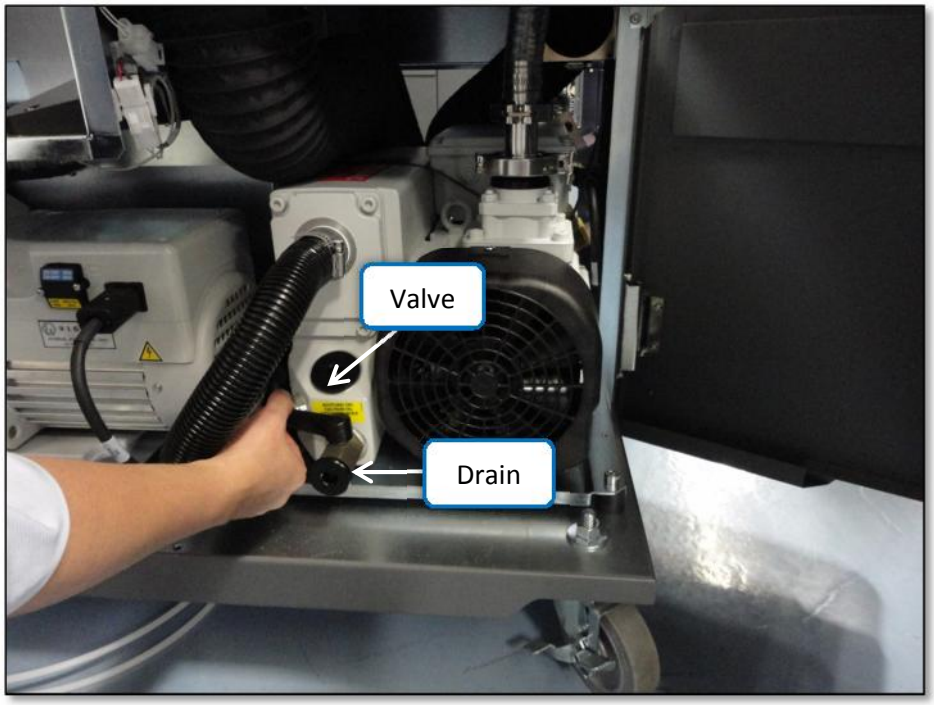


5. Open the top fill cap on the backing pump.



6. Open the bottom oil cap and drain the oil into a tray or container.
7. Replace the bottom oil cap. Pour HE-100 type vacuum oil into the top end fill hole using a funnel until the level is ¾ full in the window.
8. Replace top cap. Be careful not to over-tighten to prevent leaking.

9. Open the valve on the interface pump and drain the oil into a tray or container.



10. Refill the oil using a funnel. Fill to approximately ¾ full using interface pump sight glass as a guide (behind hand in picture above).
11. Replace the cap. Be careful not to over-tighten to prevent leaking.
12. Close right side door and door to bottom compartment. Close front access door.
13. Start the vacuum pumps and wait for the vacuum level to return to specification.

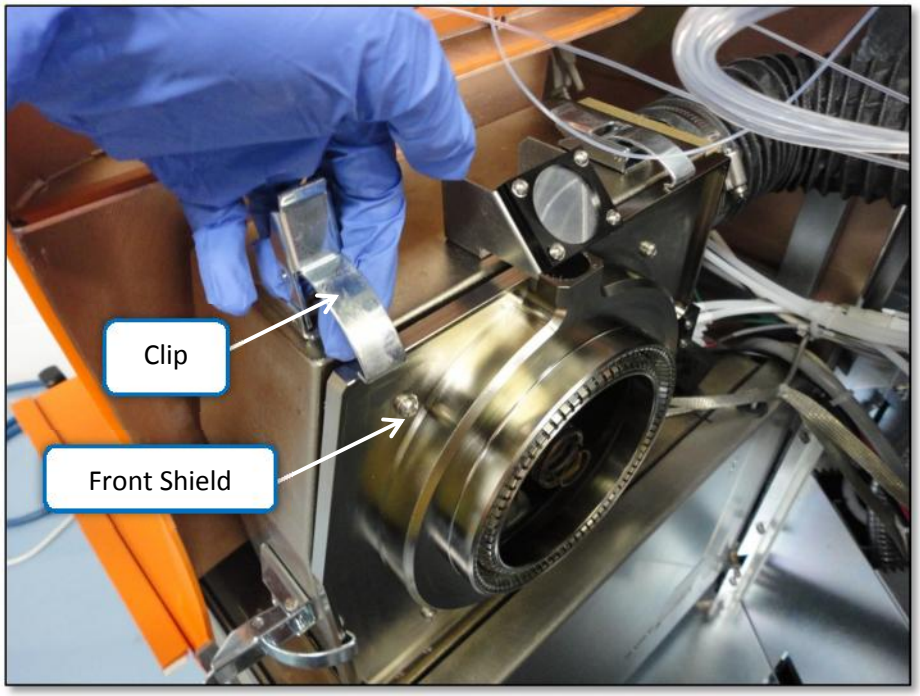
Unscheduled Maintenance

Replacement of Load Coil

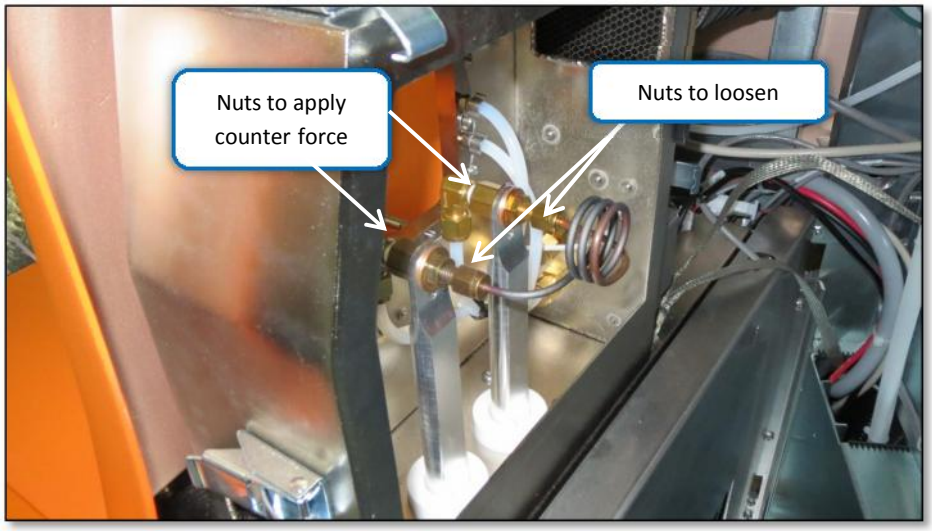
If the load coil shape is warped or if any deposits or damage exist, the load coil needs to be replaced.

1. Remove the Torch Assembly.
2. Open the Front Access Door.

3. Remove the Front Shield by undoing the clips on all 4 sides and then lifting off.

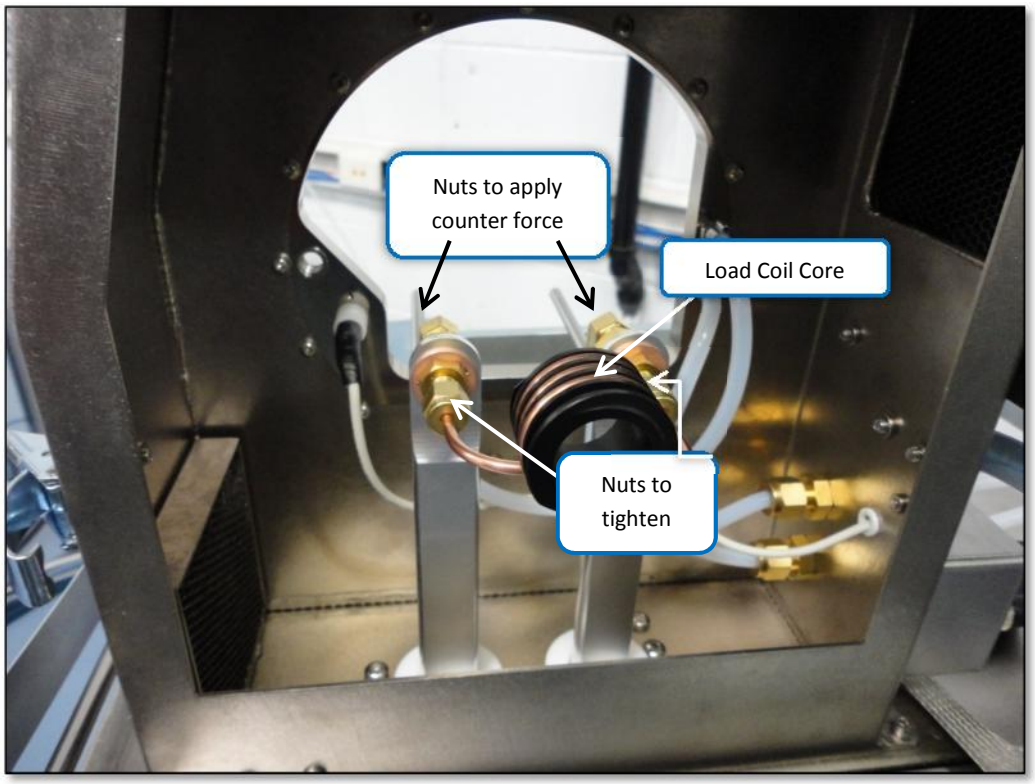


4. Using a wrench, loosen the 2 nuts that hold the Load Coil in place. It may be necessary to apply counter force on the larger nuts.



6. Install the new Load Coil. Make sure that the Load Coil Core is in place before installing.

7. Tighten nuts with the wrench.



8. Remove the Load Coil Core.
9. Replace the Front Shield and clip in place.
10. Install the Torch Assembly.
11. Check that the Torch is centered in the Load Coil (in section “Checking the Torch Alignment” above).

Procedure for Expected Power Outages

When a power outage is scheduled for the facility, the CyTOF 2 instrument needs to be properly shut down. Follow the steps below to shut down prior to the power outage and restart after power is restored.

CyTOF Shutdown

1. Ensure that the system is connected to the argon supply (the vacuum chamber will be filled with argon when vented).
2. Press Vacuum Off button on the side panel (on the left side of the instrument below the circuit breakers).
3. Wait for 10 minutes. (Initially the turbo-pumps will be heard to be slowing down gradually. Then the venting valve will open and the chamber will be filled up with argon through the purge valve slowly at controlled pressure).
4. After the turbo-pumps have slowed down, the power can be shut off. At this point, the reading on the vacuum gauge controller VGC402 will be in ~ Torr range, as opposed to the <1E-6 Torr operational pressure and ~ 1E-6 - 1e-4M Torr when the turbo-pumps are slowing down but the venting valve is not yet open. It will open after turbo-pumps are slowed down completely.
5. Let flush with argon for 10 minutes.
6. Switch OFF the circuit breakers in this sequence AC Outlets, Backing Pump, RF Generator and System.
7. Leave argon supply ON (if one wants to save on argon and can compromise on time taken for vacuum to build up, you can turn the argon supply OFF).

CyTOF Startup

1. Ensure that the Argon supply was left ON during the shutdown. If not then, open the Argon gas supply and wait for 2 hrs.
2. Switch ON the circuit breakers in this sequence: System, RF Generator, Backing Pump & AC Outlets.
3. After the system is powered up, the RFG Test LED on the instrument control panel will be red. This is normal. Just press RFG Test button on the side panel (below the circuit breakers), to turn it off.
4. Press Vacuum ON button:VG1 will come on first, then TP1 and TP2 will come up after ~ 6 minutes or so, and finally VG2 will come on after up to >30 minutes.
5. Chiller may have to be manually switched off.

When the system is in a stand-by mode, all LEDs mentioned above are lit.

Wait for the vacuum reading (on the VGC402) to reach 1E-6 Torr before attempting to start plasma.

NOTE: Vacuum readings can be found behind the lower door on the left side of the instrument.

Chapter 7
Safety

Introduction

This document describes general practices designed to aid you in safely operating the CyTOF®2 and its accessories.

This advice is intended to supplement, not supersede, the normal safety codes in your country. The information provided does not cover every safety procedure that should be practiced. Ultimately, maintenance of a safe laboratory environment is the responsibility of the operator and the operator's organization.

Please review all manuals supplied with the CyTOF®2 and accessories before you start working with the instrument to prevent personal injury or damage to the instrumentation. Carefully read the safety information in this chapter and in the other manuals supplied. When setting up the instrument or performing analyses or maintenance procedures, strictly follow the instructions provided.

Symbols

The warnings provided in this manual must be observed during operation and maintenance of the CyTOF®2.

Symbol		Description
	Warning Symbol	General warning symbol. Indicates a hazardous situation, that, if not avoided, could result in death or serious injury.
	Radio Frequency Radiation Symbol	

	Hot Surface Symbol	Hot surface warning sign, do not touch. Potential for personal injury.
	Ionizing Radiation Symbol	Radiation hazard warning symbol.
	Compressed Gas Hazard	Any product, material or substance contained under pressure, including compressed gas, dissolved gas or gas liquefied by compression or refrigeration

Table 7.1. Hazard Symbols. This table summarizes the hazard symbols that may be observed in this manual as well warning labels on the CyTOF ®2 instrument.

General Safety Guidelines

This section describes some general laboratory safety guidelines. For additional information, we recommend The CRC Handbook of Laboratory Safety (Furr, 1990) and Prudent Practices for Handling Hazardous Chemicals in Laboratories (National Research Council, 1981).

Adherence to the following safety precautions should be maintained at all times when setting up, operating, and maintaining the CyTOF system.

- Never view the ICP torch directly without protective eyewear such as safety glasses. This is a bright source of ultraviolet radiation. Safety glasses with side shields will provide an extra margin of safety as well as mechanical protection for your eyes. Potentially hazardous ultraviolet radiation may be emitted.
- ICP-based instruments generate high levels of radio frequency energy within the RF power supply and the torch box. The RF energy is potentially hazardous if allowed to escape. Safety devices and safety interlocks should not be bypassed or disconnected.
- The power supplies of the CyTOF instrument are capable of generating potentially lethal voltages. Store the removable instrument handle separately from the instrument. No maintenance should be performed by anyone other than a DVS Sciences Service Specialist or by the customer's own DVS-trained and appropriately certified maintenance personnel.
- Do not allow smoking in the work area. Smoking is a source of significant contamination as well as a potential route for ingesting harmful chemicals
- When installing or moving the instrument contact a DVS Sciences field service engineer for assistance. The total weight of the instrument is 295 kg (650 lbs).
- Food should not be stored, handled, or consumed in the work area.

Environmental Conditions

Refer to the “Preparing Your Laboratory for the CyTOF ®2 Mass Cytometer” guide for the recommended environmental conditions.

Laboratory Ventilation

Toxic combustion products, metal vapor, and ozone can be generated by the CyTOF system, depending upon the type of analysis. Therefore, an efficient ventilation system must be provided for your instrument. When the plasma is on, hot gases are vented through two exhaust vents located at the back of the instrument. Detailed information on exhaust vents are described in the “Preparing Your Laboratory for the DVS Sciences Inc. CyTOF ®2 Mass Cytometer” guide.

Warning! The use of CyTOF instruments without adequate ventilation to outside air may constitute a health hazard. Extreme care should be taken to vent exhaust gases properly.

Warning! CyTOF instrument is designed for analysis of fixed/permealized, non-live cells only. Under normal operation, cells are completely combusted in the ICP. High levels of UV radiation inside the torch box are significantly above the lethal levels for most of single airborne cells. However, in the event of plasma shutdown, the undigested portion of a sample can enter the torch box exhaust gases. Extreme care should be taken to vent exhaust gases properly.

Electrical Safety

The CyTOF series products have been designed to protect the operator from potential electrical hazards. The following section describes recommended electrical safety guidelines.

Symbols	Title	Description
	Electric Shock Hazard Symbol	This sign indicates high electricity, electric shock. Electrical machines and/or equipment in the vicinity. You may suffer severe injuries or even death.
	Earth-Ground Symbol	The earth-groud symbol represents the any terminal which is intended for connection to an external conductor for protection against electric shock or the terminal of a protective earth.

Table 7.2. Electrical Hazard Symbols. This table represents the symbols you will see on the CyTOF 2 instrument and its accessories.

Water lines should be located away from electrical connections. Condensation and potential leaks may create an unsafe environment in the proximity of electrical connections

Warning! If this equipment is used in a manner not specified by DVS Sciences Inc., the protection provided by the equipment may be compromised.

Warning! Lethal voltages are present at certain areas within the instrument. Installation and internal maintenance of the instrument should be performed only by a DVS Sciences field service engineer or similarly authorized and trained by DVS personal.

- When the instrument is connected to line power, opening instrument covers is likely to expose live parts.
- High voltages can still be present even when the power switch is in the off position.
- Disengage the circuit breakers before performing any service or maintenance on the cones or torch.

Warning! Before performing any maintenance, turn off the RF generator power supply and allow for 2 minutes of cool down prior to accessing the ICP torch, load coil and cones.

Warning! Prior to disengaging the torch box from the vacuum chamber switch off the RF generator power using the breaker, located at the left rear of the CyTOF instrument and at the right rear of the CyTOF 2 instrument.

- Capacitors inside the instrument may still be charged even if the instrument has been disconnected from all voltage sources.
- The instrument must be correctly connected to a suitable electrical supply (see Preparing your Laboratory for the CyTOF ®2 Mass Cytometer for further details).
- For 50 Hz installations, a means of electrically grounding the instrument must be available.
- The power supply must have a correctly installed protective conductor (earth-ground) and must be installed or checked by a qualified electrician before connecting the instrument.

Warning! Any interruption of the protective conductor (earth-ground) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous.

- Connect the instrument to a correctly installed line power outlet that has a protective conductor connection (earth-ground).
- Do not operate the instrument with any covers or internal parts removed.
  Do not attempt to make internal adjustments or replacements except as directed in the user manual.
- Disconnect the instrument from all voltage sources before opening it for any adjustment, replacement, maintenance, or repair.
- Use only fuses with the required current rating and of the specified type for replacement.
- Do not use makeshift fuses or short-circuit the fuse holders.
- If there are any signs that the instrument is no longer electrically safe for use, make the instrument inoperative and secure it, with a lockout, against any unauthorized or unintentional operation. The electrical safety of the instrument is likely to be compromised if the instrument:

- Shows visible damage
- Has been subjected to prolonged storage under unfavorable conditions
- Has been subjected to severe stress during transportation.

Warning! The radio frequency (RF) power supply driving the plasma torch provides up to 1.6 kW. The resulting voltages may cause extensive burns - even death. Under no circumstances should you attempt any physical adjustments of the plasma torch when it is operating. The instrument must be operated with the RF generator in the locked position at all times.

Warning! Do not attempt to defeat the safety interlocks. This would place the operator's safety at risk. All interlocks must be engaged before you ignite the plasma.

Chemical Safety

In this section, we have provided some general safety practices that you should observe when working with any chemicals. The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable Material Safety Data Sheets (MSDS) provided by the manufacturer or supplier.

Symbol		Description
	Poison Hazard Symbol	Very hazardous to health when inhaled, swallowed or when they come in contact with the skin. May even lead to death. Hazardous materials, toxic or very toxic materials
	Corrosive Materials Hazard	Potential personal injury hazard. Includes caustic and acid materials that can destroy the skin and eat through metals.

Table 7.3. Chemical hazard symbols. This table summarizes the chemical hazard symbols that you may encounter working with CyTOF reagents.

When handling any chemical the following safe-handling guidelines should be strictly observed:

- Use, store, and dispose of chemicals in accordance with the manufacturer's recommendations and regulations applicable to the locality, state/ province, and/or country.
- When preparing chemical solutions, always work in a fume hood that is suitable for the chemicals you are using.
- Conduct sample preparation away from the instrument to minimize corrosion and contamination.
- Clean up spills immediately using the appropriate equipment and supplies and follow the appropriate MSDS guidelines
- Do not put open containers of solvent near the instrument.
- Store solvents in an approved cabinet (with the appropriate ventilation) away from the instrument.

Warning! Some chemicals used with this instrument may be hazardous or may become hazardous after completion of an analysis.

Warning! Venting for fumes and disposal of waste must be in accordance with all national, state/provincial and local health and safety regulations and laws.

- Wear the appropriate personal protective equipment (PPE) at all times while handling chemicals. Use safety glasses (with side shields), goggles, or full-face shields, according to the types of chemicals you will be handling.

Warning! Wear suitable protective clothing, including gloves specifically resistant to the chemicals being handled.

Warning! Wear protective clothing and gloves. Some reagents are readily absorbed through the skin.

Drain Vessel

A drain vessel is supplied with the CyTOF®2 system. The vessel is made of HDPE and is used to gather the effluent from the Flow Injection Valve of the sample introduction system. For safe operation of your system, you should properly install and maintain the drain vessel and drain tubing. Waste disposal procedures **must be in accordance with all national, state/provincial and local health and safety regulations and laws.** Drain vessels may contain flammable, acidic, caustic, or organic solutions, cells debris and small amounts of the elements analyzed.

Warning! It is necessary to follow appropriate waste segregation guidelines in order to prevent effluents from

Warning! Never place the vessel in an enclosed cabinet. Doing so may result in a build-up of hazardous gases.

Warning! Do not use a glass drain vessel. A glass drain vessel may break and spill toxic or corrosive liquids.

- Place the drain vessel in an area that is visible to the operator, who can observe the level of collected effluent and empty the vessel when necessary.
- Check the condition of the drain tubing regularly to monitor deterioration. Organic solvents deteriorate the tubing more quickly than aqueous solutions. When the tubing becomes brittle or cracked, replace it.

Empty the drain bottle regularly. Disposal of waste must be in accordance with all national, state/provincial and local health and safety regulations and laws.

Pressurized Gas Safety

Safe Handling of Gas Cylinders

Ar gas used with CyTOF systems is normally stored in liquid argon tanks or pressurized containers. Carefully use, store, and handle compressed gases in cylinders. Gas cylinders can be hazardous if they are mishandled. Argon is neither explosive nor combustible.

Contact the gas supplier for a MSDS containing detailed information on the potential hazards associated with the gas.

Symbol		Description
	Compressed Gas Hazard	Any product, material or substance contained under pressure, including compressed gas, dissolved gas or gas liquefied by compression or refrigeration

Table 7.4. Compressed gas hazard symbols.

The following hazards are associated with pressurized containers of argon:

• Muscle strain

• Physical injury (i.e., from a bottle falling)

• Suffocation

Warning! If liquid argon is used, the gas cylinder must be fitted with an overpressure regulator, which will vent the cylinder as necessary to prevent it from becoming a safety hazard.

The following are some general safety practices for the proper identification, storage, and handling of gas cylinders.

Legibly mark cylinders to identify their contents. Use the chemical name or commercially accepted name for the gas. In North America, as in most countries, all chemical or gas storage containers must be identified by means of approved labels (i.e., WHMIS labels).

Note: See the Preparing Your Laboratory for the CyTOF ®2 Mass Cytometer guide for detailed information on the correct storage of gas cylinders.

Handling Cylinders

- Move cylinders with a suitable hand truck after ensuring that the container cap is secured and the cylinder properly fastened to the hand truck.
- Never roll or drag a compressed gas cylinder. Use a wheel cart.
- Always use a stand or safety strap while using or storing a cylinder.
- Replace the protective cap on the valve when the cylinder is not in use.Use only regulators, tubing, and hose connectors specifically approved by an appropriate regulatory agency to be used with the gas in the cylinder.
- Never lubricate regulators or fittings Do not force caps off with tools. If stuck, contact the supplier.
- Arrange gas hoses where they will not be damaged or stepped on, and where objects will not be dropped on them.
- Do not refill gas cylinders.Check the condition of pipes, hoses, and connectors regularly.
- Perform gas leak tests at all joints and seals of the gas system regularly, using an approved gas leak detection solution.
- Close all gas cylinder valves tightly at the cylinder when the equipment is turned off.

Liquid Argon Handling

Carefully inspect argon tanks prior to use

- Ensure that good ventilation is maintained in the laboratory space that will contain the liquid argon cylinders

Warning! Oxygen monitors should be installed in the laboratory to ensure that dangerous enriched oxygen environments are not created.

Warning! Liquid argon in the cylinder is maintained at extremely low temperatures. Personal protective equipment including gloves, safety glasses and long sleeved clothing should be worn when operating the cylinder.

- Cryogenic liquid cylinders contain a vacuum that helps to maintain the integrity of the liquid argon in the cylinder. This vacuum may become compromised if the following symptoms are observed:
    - The outer vessel shows signs of frosting
    - The outer vessel sweats in humid conditions
    - The pressure relief valve opens continuously until the vessel is emptied

- Never lay or store the cylinder on its side. Cylinders should be stored in a vertical position.
- Do not roll a cryogenic liquid cylinder.
- Cylinders may be moved using a cart, overhead crane or hoist.

Sample Handling and Preparation

Sample preparation for the CyTOF ®2 may require the handling of organic or corrosive solutions. MAXPAR® reagents that are used with CyTOF instruments are supplied in a solution form. Please refer to the information supplied with MAXPAR reagent Material Safety Data Sheets (MSDS) for safe handling of the reagents.

Hydrofluoric Acid

Trace amounts (< 0.1 % w/v) of hydrofluoric acid (HF) may be present in the wash-out solution. Hydrofluoric acid is toxic and extremely corrosive. It will readily burn skin and lung tissue (if the fumes are inhaled). Burns may not be immediately painful or visible. Contact with eyes could result in blindness. Do not use a glass beaker when working with HF as HF will attack the glass.

Warning! Before using hydrofluoric acid, you should be thoroughly familiar with its hazards and safe handling practices. Observe the manufacturer's recommendations for use, storage, and disposal.

Warning! For better control of contamination, dedicate laboratory reagents and consumables to use with CyTOF instrument and MAXPAR reagent only.

Other Hazards

Protection from Ultraviolet Radiation and Heat

Warning! The plasma generates high intensity ultraviolet radiation. A safety interlock is used to automatically shut off the plasma if the chamber and interface are not fully coupled. Do not defeat the interlock. Do not remove the shield which protects the sample introduction system: the shield is designed to block any residual amounts of the ultraviolet radiation.

Protection from Radio Frequency radiation

Warning! Radio Frequency Radiation: The instrument generates high levels of Radio Frequency (RF) energy, which is potentially hazardous if allowed to escape. The instrument is designed to contain the RF energy within the shielded enclosures of the torch compartment and the RF power supply. Safety interlocks prevent you from operating the system without all covers, doors, and shields in place.

Hot Surface Temperatures

Warning! Hot Surface Temperatures: The torch components, the interface and the sample introduction system components remain hot for some time after the plasma has been shut off. Allow sufficient time for these items to cool to room temperature before you handle them.

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Chapter 8
Troubleshooting

The following table presents recommended solutions for symptoms you may encounter. If additional help is required, contact technical support at support@dvssciences.com or by phone +1-855-387-2986.

Symptom	Possible Causes	Recommended Solutions
Plasma does not ignite/ plasma flickers	RFG Circuit Breaker is switched off.	Switch the circuit breaker on.
	Vacuum Levels are not achieved.	Check the "Monitor" window. VGAUGE 1 must be <1E-6 Torr and VGAUGE2 must be 6.3E-4 Torr (when plasma is not lit). If these are not met, a shutdown and restart of the instrument is required. Contact DVS Sciences Technical Support for guidance.
	Argon pressure is incorrectly set/ there is not enough argon.	Verify and adjust argon pressure on tank to around 100psi and CyTOF regulator pressure behind the instrument to around 50 psi. Also check level of argon in the tank and replace tank if necessary.
	Exhaust is out.	If the "EXHAUST" LED light is off, it indicates that there may be a problem with the exhaust fan within the building.

Symptom	Possible Causes	Recommended Solutions
Plasma does not ignite/ plasma flickers (Continued)	Chiller is not turned on.	The chiller should turn on automatically within 20 seconds after user confirms plasma start. If "CHILL" LED light is off, it indicates that the chiller has not been turned on by the software. Manually switch on the chiller in the Card Cage tab and ensure the "CHILL" LED light comes on.
	There is moisture in glassware.	Inspect glassware for moisture that may be present and interfering with plasma ignition. Completely dry glassware especially the Spray Chamber, Injector and Torch.
	Connections of gas line are incorrect.	Ensure tight and correction gas line connection on Nebulizer, Spray chamber, and Torch.
	Torch has melted	Argon flow is not maintained; check for leaks in Torch assembly and gas lines near the interface area. Check argon pressure. Check Load Coil for deposits. Replace Torch and, if necessary, Load Coil.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer (see "Instrument Setup and Preparation for Plasma Start" in Chapter 5).
	The Load Coil is not clean/has micro-punctures/spikes.	Clean the load coil so that the surface is smooth and free of debris. If necessary, such as when there are small punctures present, replace the load coil.
No signal detected during performance check	One of the above causes.	Follow the corresponding recommended solution for the cause.

Symptom	Possible Causes	Recommended Solutions
No signal detected during performance check (Continued)	Carrier reservoir is empty.	Fill the carrier reservoir with Millipore grade deionized water.
	Syringe Pump is not on.	Ensure that the Syringe Pump is running – it is indicated by the green color on the syringe status bar.
	Carrier Syringe is filled with air.	Purge the air. First, disconnect the sample capillary from the nebulizer, and then place the capillary into a vial. Click on the "Sample Intro" button in the software and enter 0.3 for flow rate. Wait until carrier solution replaces the air in the syringe. Return the sample intro flow rate to 0.045 before reconnecting the sample capillary to the nebulizer.
	Sample capillary is clogged.	Remove the sample capillary from the nebulizer and observe the droplets emerging from the capillary. If the droplets are not uniform, replace the capillary.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer (see "Instrument Setup and Preparation for Plasma Start" in Chapter 5).
	Nebulizer is damaged/clogged.	With the carrier syringe running at the normal sample introduction rate (0.045 ml/min), carefully remove the nebulizer from the nebulizer port (with all other connections intact) and check the spray with a flashlight. If the spray is absent or intermittent, clean or replace the nebulizer.
	Masses are incorrectly calibrated.	Perform mass calibration (see "Autotuning").

Symptom	Possible Causes	Recommended Solutions
No signal detected during performance check (Continued)	The analytes are not selected correctly.	Check your analytes table and make sure the analytes of interest are selected.
Unstable signals	One of the above causes.	Follow the corresponding recommended solution for the cause.
	Nebulizer is not connected properly.	Check Nebulizer Gas connection and reconnect if necessary.
	Syringe Pump has malfunctioned.	Ensure that Syringe Pump is running properly.
Low Tuning solution Signals (Tb signals <400,000 dual counts per picogram)	One of the above causes.	Follow the corresponding recommended solution for the cause.
	Heater is not on/ set to the correct temperature.	Ensure heater temperature is at 200 ^{0}C. If not at 200 ^{0}C, check for moisture in the glassware and if necessary remove to dry after shutting off plasma.
	Argon Pressure is not maintained.	Ensure steady argon supply and proper argon pressure is maintained (~100 psi on tank and ~50 psi on regulator).
	Plasma is unstable	See above for recommended solutions for plasma ignition/stability issues.

Symptom	Possible Causes	Recommended Solutions
Low Tuning solution Signals (Tb signals <400,000 dual counts per picogram) (Continued)	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer (see "Instrument Setup and Preparation for Plasma Start" in Chapter 5).
	Masses are not correctly calibrated.	Perform mass calibration (see "Auto-Tuning" in Chapter 5).
	Nebulizer and Make up gas flows are not optimal.	Perform gas optimization (see "Auto-Tuning" in Chapter 5).
	Current is not optimal.	Perform current optimization (see "Auto-Tuning" in Chapter 5).
	Detector Voltage is not optimal.	Perform detector voltage optimization (see "Auto-Tuning" in Chapter 5).
	One or more hardware parts of the instrument are not aligned properly.	See Chapter 6 "Maintenance" for proper alignment of parts and the "Auto-Tuning" section in Chapter 5 for optimizing signals.
	The glassware is not clean.	Remove the glassware according to the instructions in Chapter 6 "Maintenance" and clean.
	The interface cones are not clean.	Remove the cones according to the instructions in Chapter 6 "Maintenance" and clean.
	One or more hardware parts of the instrument need to be replaced.	Inspect all accessible hardware parts; if there are any signs (such as damage, clogging, and irremovable stains) that suggest the part is no longer functioning optimally, replace with a new one.
Oxides are >3%	Nebulizer and make up gas flows are too high	Perform gas optimization (see "Auto-Tuning" in Chapter 5).

Symptom	Possible Causes	Recommended Solutions
Unstable Signal or Oscillations from Tuning Solution	Proper exhaust level is not reached/maintained.	Ensure proper and consistent exhaust.
	Nebulizer is damaged/clogged	With the carrier syringe running at the normal sample introduction rate (0.045 ml/min), carefully remove the nebulizer from the nebulizer port (with all other connections intact) and check the spray with a flashlight. If the spray is absent or intermittent, clean or replace the nebulizer.
	Nebulizer gas line is not connected properly.	Check Nebulizer Gas connection and reconnect if necessary.
	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer (see “Instrument Setup and Preparation for Plasma Start” in Chapter 5).
	Syringe Pump has malfunctioned.	Ensure that the Syringe Pump is running properly.
No Signal from Sample	Sample is not loaded into the sample loop.	Ensure the sample is injected from the syringe into the sample loop.

Symptom	Possible Causes	Recommended Solutions
No Signal from Sample (Continued)	Sample is not present.	It is highly recommended that users add 0.1X calibration beads with the sample as an internal standard. (Refer to Product Insert for usage instructions). If the beads are present but the cells are not, it indicates the absence of cells in the sample itself. If both the beads and the cells are not visible to the CyTOF, there could be problems with one or more parts of the instrument that need to be addressed before continuing acquisition (see below).
	One or more parts of the instrument are causing the problem.	Refer to "No Signal detected during performance check" for possible causes and recommended solutions.
Sample leaking from carrier line or valve	Sample capillary is not positioned correctly in the nebulizer sample inlet.	Make sure that the sample capillary ends at the tapered portion of the sample inlet of the nebulizer (see "Instrument Setup and Preparation for Plasma Start" in Chapter 5). With leakage, the capillary is often too far in and has bent. Trim capillary or replace if too damaged.
Cells are indistinct from each other (streaky signals)	Concentration is likely too high.	Immediately stop the acquisition when there are more than 3 continuous refreshes of "streaky signals" to prevent detector damage. Look for the marker(s) that produces this continuous streak of signals.

Symptom	Possible Causes	Recommended Solutions
Cells are indistinct from each other (streaky signals) (Continued)	Too many cells are introduced	Dilute the sample with DIW. Concentration of cells introduced should be 1E6/mL, at maximum. Lower cell concentrations improve signal resolution.
	The concentration of intercalator is too high	Before the acquisition, wash the sample once more with DIW. If the signals are still too strong, wash once again with DIW.
	The source of streaky signals is one of markers used.	Make sure the antibodies are titrated prior to the experiment, ideally with the cell type of interest.